EVALUATION OF A NOVEL XYLANASE AND ALGAL BETA-GLUCAN ON BROILER PERFORMANCE, ENERGY DIGESTIBILITY, AND IMMUNE STATUS

A Thesis

by

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ABSTRACT

The objective of this research was to determine the effects of a novel xylanase and algal β-glucan (ABG) when included in broiler diets on broiler performance, ileal digestibility of energy (IDE), and immune status. Three experiments were conducted to evaluate the effectiveness of ABG during a challenge and non-challenge setting. In Eexperiment 1, inclusion of ABG at 750 g/MT increased ($p<0.05$) d 14 average broiler body weights (BW) as compared to the control diet; ABG had no impact on mortality corrected feed conversion ratio (FCR) or relative organ weights. In Experiment 2, inclusion of ABG at 250 g/MT increased ($p<0.05$) d 10 BW of as compared to the control group prior to *Eimeria* challenge; inclusion of ABG at 250 and 750 g/MT also increased ($p<0.05$) BW 7 and 10 d post-challenge as compared to the non-challenged control group. The inclusion of ABG at 250 and 750 g/MT reduced ($p<0.05$) d 10 FCR as compared to the control group prior to *Eimeria* challenge. No improvements to performance, intestinal lesion score, or oocyst output were observed with ABG inclusion as compared to the control diet. In experiment 3, inclusion of ABG had no benefit to performance during a Newcastle Disease Virus vaccination program; however, inclusion of ABG at 250 g/MT increased ($p<0.05$) Newcastle Disease specific antibody titers.

Two experiments were conducted to evaluate the effectiveness of xylanase in diets containing wheat and DDGS at 30 and 15%, respectively, when pelleted at 82 and 92°C, on performance and IDE. In Experiment 1, the inclusion of xylanase at 1000 and 2000 U/kg in diets manufactured at 82°C increased ($p<0.05$) d 28 BW as compared to the control diet; inclusion of xylanase at all dosage levels reduced ($p<0.05$) d 38

cumulative FCR as compared to the control diet. Inclusion of xylanase at 250, 500, and 2000 U/kg increased ($p<0.05$) d 38 IDE as compared to the control diet. In Experiment 2, the inclusion of xylanase in diets pelleted at 92°C had no impact on broiler BW throughout the experiment; however, inclusion of xylanase at all dosage levels reduced ($p<0.05$) d 21 cumulative FCR. Inclusion of xylanase at doses of 500 U/kg and higher increased ($p<0.05$) IDE as compared to the control diet.

A final experiment was conducted to evaluate the effectiveness of xylanase in an energy reduced, corn-SBM diet on performance, IDE, digestive tract parameters, and processing parameters. Inclusion of xylanase had no impact on BW throughout the experiment; however, inclusion of xylanase at 250 U/kg in the energy reduced diet reduced ($p<0.05$) cumulative FCR to levels comparable to the positive control. Inclusion of xylanase at 1000 U/kg reduced ($p<0.05$) ceca DM content and jejunum viscosity levels as compared to the energy reduced diet; inclusion of xylanase at 2000 U/kg reduced ($p<0.05$) pancreas weights as compared to other doses of xylanase inclusion. Inclusion of xylanase at 250 U/kg increased ($p<0.05$) breast meat yield as compared to the negative control at the conclusion of the experiment.

These data confirm the benefits of novel feed additives to improve growth performance and feed efficiency. Inclusion of an algal derived β-glucan improved early broiler performance and immune response as evidenced with improved performance during an *Eimeria* challenge and increasing Newcastle Disease Virus specific antibody titers. Inclusion of exogenous xylanase increases IDE, resulting in reductions in FCR.

DEDICATION

This thesis is dedicated to those who stimulated and supported my interest in agriculture and shown their support throughout this entire process, from show barn to research to the start of my career.

To my grandparents, thank you for laying the foundation and instilling the importance of agriculture into my parents and for your love and support throughout my life. You have always been there as the backbone to support my interests, no matter what they are.

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CHAPTER I

INTRODUCTION

Feed accounts for the largest cost of production to broiler integrators. Because of this, nutritionists strive to lower feed costs through a variety of measures such as probiotics, prebiotics, exogenous enzymes, and alternate ingredients, while maintaining or improving the current performance status of broilers. The use of feed additives such as exogenous enzymes and β-(1,3)-(1,6)-glucans have been extensively reviewed over the past two decades. The commercialization of exogenous enzymes is estimated to save global animal agriculture $3-5 billion annually (Cowieson et al., 2010). The use of xylanase and phytase is estimated to have a feed cost savings of $10-12 per tonne (Cowieson et al., 2010). It is important to continue to analyze new sources for xylanase and β-glucan products as new generation phytases have consistently outperformed the original phytases that were initially used. Improving efficiency will continue to reduce diet costs, and thus consumers, money.

The demand for limited and reduced use of antibiotics in poultry production has caused an increase in use of alternative supplements to benefit immune function and growth performance. One alternative that has been extensively reviewed is the inclusion of β-glucans in poultry diets. Beta-(1,3)-(1,6)-glucans are a structural component of the cell wall of many bacteria, fungi, and yeasts. (Jorgensen and Robertsen, 1995) Variations in β-glucan structure, molecular weight, degree of branching, and intermolecular association from varying sources results in different physiological functions (Bohn and BeMiller, 1995; Kulicke et al., 1997; Pins et al., 2005a, b; Volman et al., 2008). Immune modulation has been attributed to β-glucan due to its highly branched structure and insolubility (Vetvicka and Vetvickova, 2007; Zhang et al., 2008; and Cox et al., 2010b).

Numerous articles have detailed the effects of yeast cell wall β-glucans and their immune modulating effects and evaluating broiler performance as compared to antibiotic growth promoters as an alternative form for disease control. Coccidiosis and Newcastle Disease are very costly diseases to the poultry industry (CIDRAP, 2003; Dalloul and Lillehoj, 2006; Shirley et al., 2006). *Eimeria* species are known to invade the intestinal tract of animals, disrupting digestion and impeding absorption, causing performance losses to the host. Yeast β-glucans have been observed to improve immune function and performance in broilers (Zhang et al., 2012), decrease fecal oocyst counts (Shanmugasundaram et al., 2013), and reduce intestinal lesions and severity (Cox et al., 2010b). An increase in virus specific antibody titers has also been observed in Newcastle vaccinated broilers fed β-glucans (An et al., 2008). It is suggested that the immune status of the animal may play a role in performance responses of broilers fed diets supplemented with β-glucan (Chae et al., 2006; Cox et al., 2010a).

Although β-(1,3)-(1,6)-glucans have been shown to modulate immune responses and benefit gut health in broilers, β-(1,3)-(1,4)-glucans are classified as non-starch polysaccharides (NSP) which hinder growth performance. Grains such as wheat, barley, and dried distillers grains with solubles (DDGS) contain high concentrations of NSP including β-glucan, arabinose, xylose (arabinoxylan), cellulose, and other cellulitc and non-cellulitic polysaccharides (Henry, 1987; Slominski et al., 2000; Widyaratne and

Zijlstra, 2007). The presence of NSP, which are indigestible by monogastric species, at large concentrations in poultry diets results in reductions in nutrient utilization (Meng et al., 2005). The antinutritive properties of NSPs include reductions in nutrient digestibility, reduced AME values, increases in intestinal viscosity, and overall reductions in broiler performance (Pettersson and Aman, 1988; Annison, 1993; Choct, 2006). The increase in viscosity in the small intestine causes a decrease in contact between digestive enzymes, leading to a decrease in nutrient absorption and broiler performance (Choct et al., 1995).

Pelleting wheat diets at temperatures over 60°C has been reported to increases diet and digesta viscosity (Abdollahi et al., 2010). Increases in previously encapsulated NSP have been observed when pelleting wheat based diets at elevated temperatures, resulting in overall decreases in performance parameters (Coweison et al., 2005; Abdollahi et al., 2010). Abdollahi et al. (2010) also reported a decrease in ileal digestibility of starch and protein in broilers when manufacturing wheat based feeds at elevated temperatures. Increasing conditioning temperature in corn based diets has also been shown to impact broiler performance by increasing FCR, although starch digestibility was unaffected (Abdollahi et al., 2010).

The use of supplemental carbohydrases in broiler diets to improve performance and decrease the effect of NSPs is widely accepted. Carbohydrases degrade high molecular weight polysaccharides such as arabinoxylan to simple sugars, oligosaccharides, and low molecular weight polysaccharides (Slominski et al., 1993; Castanon et el., 1997). Xylanase cleaves and hydrolyzes the xylose backbone of arabinoxylans, allowing access to entrapped nutrients by the bird and increasing nutrient utilization (Choct and Annison, 1992; Coweison et al., 2005; Meng et al., 2005). Xylanase reduces intestinal viscosity by degradation of soluble arabinoxylans leading to improvements in nutrient utilization and performance uniformity between flocks (Choct and Annison, 1992; Choct et al., 1999; Coweison et al., 2005).

It has been suggested that undigested nutrients in corn based diets, such as starch, may be hindered by antinutrient factors in corn. Although low in NSP, an estimated 400-450 kcal of energy per kg of diet goes undigested by broilers in corn-SBM diets (Cowieson, 2010) representing a potential energy source for growth. The loss of energy to undigested fat, starch, and protein presents an opportunity to use exogenous enzymes to increase available nutrients to poultry (Cowieson, 2010). Additionally, energy can be removed from diets through the reduction of fat and increasing corn content of the diet (Masey O'Neill et al., 2012). Reducing energy content is typically detrimental to broiler performance, (Cowieson et al., 2010; Masey O'Neill et al., 2012; Singh et al., 2012; Williams et al., 2014), ileal digestibile energy (Cowieson et al., 2010; Yegani and Korver, 2013), and carcass fat pad weights (Williams et al., 2014). The use of xylanse in energy reduced diets has resulted in improvements to FCR (Cowieson et al., 2010; Masey O'Neill et al., 2012; Williams et al., 2014), increases in ileal digestibility of energy (Cowieson et al., 2010; Nian et al., 2011a; Yegani and Korver, 2013), amino acids, and dry matter (Yegani and Korver, 2013).

Advances in enzyme technology allow for products to be added to the mash feed in the mixer prior to pelleting. Products can be pelleted within the feed at high temperatures due to a protective coating around the enzyme. Pelleting wheat diets for broilers supplemented with xylanase at elevated temperatures up to 80°C has been reported to maximize broiler performance compared to more extreme temperatures (Silversides and Bedford, 1999), although Cowieson et al. (2005) reported benefits to performance in diets manufactured at 90°C.

The objective of this series of experiments is to evaluate two novel feed additives on immune status and performance and determine their efficacy in poultry diets to reduce feed costs. The first series of experiments will investigate the effects of an algal β-1,3-glucan (ABG) product (Algamune ZPC™, Algal Scientific Corporation) on broiler performance, oocyst output following an *Eimeria* challenge, and antibody titer levels following Newcastle Disease vaccination in a series of three experiments. An extensive review of literature indicated no previous experiments evaluating an algae β-glucan on poultry performance and immunological effects. A second series of experiments will evaluate an experimental xylanase on broiler performance and ileal digestible energy.

CHAPTER II

LITERATURE REVIEW

Immune Modulation Through the use of Feed Additives

Until the last decade, the poultry industry has relied heavily on chemotherapeutic approaches in the prevention and control of disease outbreaks (Cox and Dalloul, 2010). Antibiotics have been utilized as forms of growth promotants in production agriculture at sub-therapeutic dosage levels to decrease flock variability (Miles et al., 1984), improve digestion and absorption of fats and carbohydrates (Eyssen and DeSomer, 1963), and to improve growth performance and overall health of the animal (Cox and Dalloul, 2010; Huyghebaert et al., 2011). The practice of using antibiotics as growth promoters for livestock and poultry was banned in 2006 in the European Union (Castanon, 2007) citing government and consumer concerns over chemical residues retained in meat and development of antibiotic resistance in pathogens to these products (Cox and Dalloul, 2010). With increasing pressure in the United States to follow suit (Dibner and Richards, 2005; Castanon, 2007), the search for alternatives to antibiotic growth promoters is warranted.

Modulation of the Immune System. One alternative to antibiotic growth promoters that has emerged is the use of β-glucans. Just as in humans, broiler immune systems are underdeveloped and inefficient protecting its host at the beginning stages of life (Cox et al., 2010a). Due to their ability to stimulate the immune system, β-glucans are classified as biological response modifiers (Soltanian et al., 2009; Cox and Dalloul, 2010; Cox et al., 2010). Because β-glucans are not endogenous to animals, the immune system will not recognize β-glucan structures when introducing supplemental β-glucan (Soltanian et al., 2009; Cox and Dalloul, 2010). The newly introduced structures are recognized as foreign (Soltanian et al., 2009), and thus activate innate immunity within the host animal as the first barrier of defense against the new threat (Cox and Dalloul, 2010). Cellular receptors such as Dectin-1, complement receptor 3, lactosylceramide, scavenger receptors, and toll-like receptors have been associated with the activity of β-glucans role as an immune modulator (Brown and Gordon, 2003). Increased cellular activity through phagocyosis and destruction of pathogens occurs with β-glucan interaction with cell receptors (Gantner et al., 2003).

Phagocytic cells are the most important defense mechanisms to an animal's innate immunity (Cox and Dalloul, 2010). Beta-glucans, both soluble and insoluble, trigger innate immunity via recognition by receptors on the surface of cells used to recognize pathogenic infections (Brown and Gordon, 2003 and 2005; Cox and Dalloul, 2010). Macrophages, monocytes, heterophils, langerhans cells, neutrophils, dendritic cells, eosinophils, endothelial cells, epithelial cells, fibroblasts, and natural killer cells all contain β-glucan receptors, that, when triggered, protect the host organism from invading pathogens (Ahren et al., 2001; Kougias et al., 2002; Lowe et al., 2002; Brown and Gordon, 2003 and 2005; Soltanian et al., 2009). These receptors include CR2, LacCer, SR, Dectin-1, and TLR2 (Soltanian et al., 2009). Following activation of innate immune cells and phagocytosis of the target pathogen, a response is stimulated in adaptive immune cells from pathogen specific antigens (Cox and Dalloul, 2010). Following interaction with an invading pathogen, macrophages will produce iNOS, begining a series of reactions producing nitric oxide and toxic derivatives, allowing macrophages the ability to destroy pathogens (Tizard, 2009). Beta-glucan induce activated macrophages in poultry to produce nitrite, enhancing the ability of macrophages to kill foreign pathogens (Guo et al., 2003; Cox et al., 2010a). Activated innate immune cells that recognize a pathogenic infection not only stimulate an immediate immunity, but also an adaptive immune response to safeguard from a future pathogenic infection (Lee and Iwasaki, 2007).

The second form of immunity available to the bird, adaptive immunity, offers antigen specific, memory protection to an organism providing increased protection against recurring infection (Cox and Dalloul, 2010). Key to adaptive immunity are bursa- and thymus-derived lymphocytes (Cox and Dalloul, 2010). These lymphocytes become effector cells within the spleen and other secondary lymphoid organs and tissues where they will encounter pathogens (Dalloul and Lillehoj, 2006). Bursa-derived lymphocytes produce antigen specific anitbodies, or immunoglobulins (Ig), when the body is stimulated by pathogens (Cox and Dallouil, 2010). Broilers possess three classes of immunoglobulins, each playing a specific role in immune response: IgM, located on the surface of bursa-derived lymphocytes (B-cells) and the first antibody detected during a primary immune response; IgG, the most abundant immunoglobulin within broilers and primary lymphocyte of a secondary immune response; and IgA, the immunoglobulin associated with mucosal immunity (Sharma, 2003; Cox and Dalloul, 2010). It was suggested by Zhang et al. (2008) that β-glucans stimulate gut associated lymphoid tissue to secrete IgA, enhancing mucosal immune function. Thymus-derived lymphocytes (T-cells) modulate cell mediated immunity (Cox and Dalloul, 2010). Just as bursa-derived lymphocytes, thymus-derived lymphocytes can be categorized as helper T cells or cytotoxic T cells (Cox and Dalloul, 2010). Helper T cells activate bursa and thymus lymphocytes through multiple cytokines. Helper T cells are divided into two sub-types: Type 1 helper T cells, which produce cytokines encouraging inflammation and activate T lymphocytes; and Type 2 helper T cells, which stimulate bursa-derived lymphocyte growth and the production of antibodies (Kaiser and Staheli, 2008; Cox and Dalloul, 2010). Both Type 1 and Type 2 helper T cells inhibit the other, with suppression of Type 1 cells enhancing immunity to extracellular pathogens and suppression of Type 2 cells to build immunity to intracellular pathogens (Kaiser and Staheli, 2008; Cox and Dalloul, 2010). Cytotoxic T cells assist in the recognition and destruction of cells overcome by endogenous pathogens (Dalloul and Lillehoj, 2006). Because β-glucans function to stimulate the products of immune organs, it has been suggested that broiler diets containing β-glucan products may exhibit increases in primary and secondary lymphoid organ size (Guo et al., 2003; Zhang et al., 2008).

Beta-Glucans as Alternatives to Antibiotic Growth Promoters**.** The cell walls of bacteria, mushrooms, fungi, algae, yeasts, and cereal grains are comprised of high concentrations of β-glucans (Zekovic and Kwiatowski, 2005; Cox and Dalloul, 2010; Cox et al., 2010). A wide range of structural variation exists amongst β-glucans due to the numerous sources they compose (Cox and Dalloul, 2010). Differences in solubility, primary structure, molecular weight, degree of branching, and polymer charge results in a wide range of physiological function (Bohn and BeMiller, 1995; Zhang et al., 2005;

Leung et al., 2006; Cox and Dalloul, 2010) and large variation between beneficial effects of products containing β-glucans from different sources. Vetvicka and Vetvickova (2007) stated that of all configurations of β-glucan, β-(1,3)-(1,6)-D-glucans are the most effective immune stimulators. These structures are composed of β-linked (1,3) glycopyranosyl backbones and β-linked (1,6) side chains, (Figure 2-1), varying in concentration from source to source (Harada and Ohno, 2008). The most active β-glucans have been noted to have a degree of branching of either 0,2 to 0,33 (1 to 5 or 1 to 3 backbone residues, respectively) (Miyazaki et al., 1979; Bohn and BeMiller, 1995; Novak and Vetvicka, 2008) and of high molecular weight (Soltanian et al., 2009). Length of the main chain and thus the activity of β-glucans are also contributed to the variety of types and sources of β-glucans available (Wagner et al., 1988; Jamas et al., 1991; Bohn and BeMiller, 1995; Yoshitomi et al., 2005; Leung et al., 2006).

Although β-glucans are used in broiler diets to modulate the immune system, the overall goal of any feed additive is to reduce diet cost through improved performance. The use of fungal derived β-glucans have been intensively studied and are suggested by Soltanian et al. (2009) of being the most effective in enhancing protective immunity from pathogens. High molecular weight, branched β-(1,3)-glucan compose nearly 85% of yeast cell wall β-glucan, with an additional 3% of the structure composed of β-(1,6)-glucosidic interchain linkages (Manners et al., 1973).

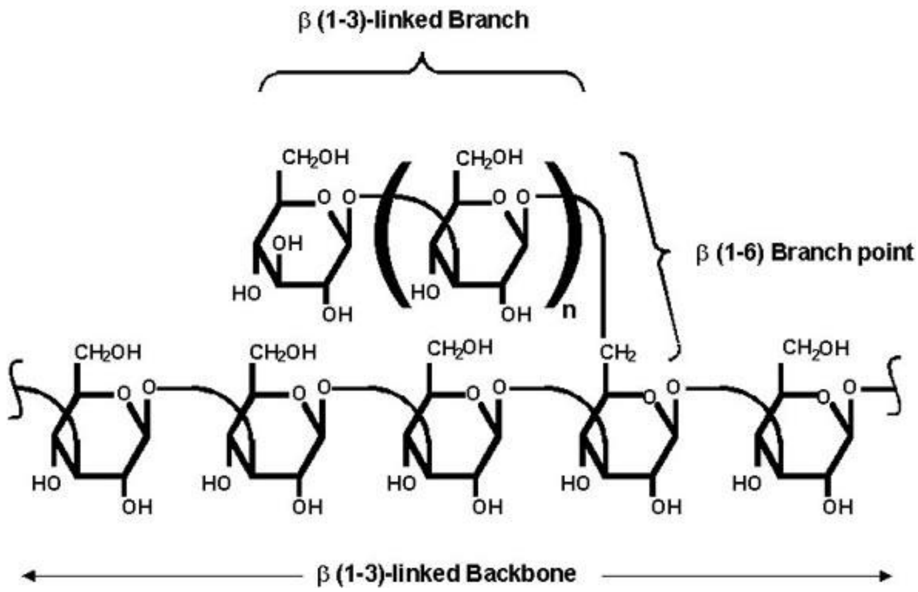

Figure 2-1. Molecular structure of β-(1,3)-(1,6)-D-glucans. (Tsukagoshi et al., 1984; Cox and Dalloul, 2010)

The use of β-glucans as an alternative to antibiotic growth promoters has been extensively studied. Rathgeber et al. (2008) compared the use of a yeast derived β-glucan (YBG) to virginiamycin in a series of three 38 d experiments. YBG was included in the diets at 40 g per MT in the starter and 20 g per MT grower and finisher diets. Performance results varied in each experiment of the publication. In the first experiment, no differences in body weights (BW) and feed conversion ratio (FCR) were observed between broilers fed YBG and virginiamycin, with both treatments exhibiting significant improvements in performance parameters compared to the control diet. This however was not the case in the second experiment, where no effects on final BW or FCR were observed between any of the dietary treatments. In the final experiment, dietary YBG increased final BW as compared to the control diet, although not different from the virgniamycin fed broilers; FCR was not affected by any dietary treatment. Cho et al. (2013) also reported equal benefits to overall performance on d 35 between supplementation of 0.1% *Agrobacterium sp.* R259 KCTC 10197B derived β-glucan and avilamycin at 40 mg/kg as compared to the non-supplemented diets. The authors concluded that both β-glucan and antibiotics play a significant role later in broiler development in terms of weight gain (Rathgeber et al., 2008). Because performance impacts were similar between β-glucan and antibiotic treated broilers, it was suggested that β-glucan products represent a feasible alternative to antibiotic growth promoters because they offer the same benefits to performance (Rathgeber et al., 2008; Cho et al., 2013).

Because of the absence of a robust immune system in adolescent birds, it is important to evaluate the early benefits of β-glucans. Although Rathgeber's group concluded similar benefits to performance of YBG to antibiotics, a study by Cox et al. (2010a) observed conflicting results. The group utilized a commercially available YBG product at doses of 0, 0.02, and 0.1% of the diet in a 14 d study. No benefits to performance were observed between diets containing YBG and the control diet. However, a dose response to BW was observed in YBG supplementation, with diets containing 0.1% YBG increasing BW as compared to the lower dosage of 0.02%. In the review by Soltanian et al. (2009), the authors state that the action of β-glucans depends on the dosage rate, with too low of a dosage having no effect and too high of a dosage negatively impacting performance. Thus it is important that optimal dosages for each source of β-glucans be identified (Vancaeneghem et al., 2000). To accomplish this task, Zhang et al. (2008) evaluated YBG dosage at 0, 25, 50, 75, 100, and 125 mg per kg of diet in a 42 d experiment. Body weights and FCR were significantly improved in broilers fed YBG at 50 and 75 mg/kg as compared to the control group; the remaining dosage rates were observed to numerically benefit performance as well, though not significantly. The authors advised that dietary supplementation of β-glucan be between 25 and 150 mg/kg. Because differences in structure and molecular weight exist between each source of β-glucan, it was suggested further research be conducted to identify specific dosage rates of each β-glucan product (Zhang et al., 2008; Vancaeneghem et al., 2000; Cox et al., 2010a).

Beta-glucan mode of action in stimulating innate and adaptive immunity was discussed previously. The benefits to broiler performance provided by β-glucans have been attributed to benefits to immune organs and cells within treated birds. In the aforementioned study by Zhang et al. (2008), benefits to immune system parameters were also observed along with performance enhancement by dietary treatment of β-glucan. The authors observed quadratic increases in cytokine levels of interleukin-1 (IL-1), IL-2, interferon γ (IFN-γ), and tumor necrosis factor α (TNF-α), which act as signal-messengers in the immune-regulating network. The highest cytokine levels were observed in β-glucan doses of 50 and 75 mg/kg, similar to the benefits to performance. In the publication by Cox et al. (2010a), supplementation of YBG to the diet reduced IL-8 expression in the intestine. IL-8 is a chemokine produced by macrophages, functioning as a chemoattractant that induces migration of target cells to areas of inflammation (Cox et al., 2010a). The reduction in IL-8 gene expression suggests β-glucan acts as an anti-inflammatory immunomodulator (Cox et al., 2010a). Contradictory to Zhang et al. (2008), Cox et al. (2010a) observed a down regulation of IFN-γ. The binding of β-glucan to the MHC compound is thought to activate monocytes and macrophages, playing a role in activation of cytotoxic microphages, helper T cells, and natural killer cells (Zhang et al., 2008). Significant increases in lymphoid organ weights were also observed with supplementation of β-glucan for the thymus, bursa, and spleen, providing further evidence that β-glucans function as immunomodulators and offers an alternative explanation for the increase in cytokine levels (Zhang et al., 2008). Cho et al. (2013) also reported increases in relative liver weight with the supplementation of β-glucan. The concentrations of peripheral blood plasma globulin, serum IgG, and intestinal secretary IgA were all elevated on d 21 and 42 of the experiment by Zhang et al. (2008). These molecules are regulated by the concentration of cytokines, number of lymphocytes, and stimulating molecules within the animal suggesting β-glucan play a role in cellular and humoral immunity (Zhang et al., 2008). The findings of Cho et al. (2013) contradict those by Zhang et al. (2008), who observed no differences in blood profiles or relative weights of the spleen and bursa with supplementation of β-glucan during the 42 d experiment.

Further evaluation of β-glucan supplementation comparing floor and cage reared birds was conducted by Chae et al. (2006). No impact of β-glucan was observed on starter performance when comparing open floor to cage reared birds. However, open floor reared birds were observed to have increased BW and feed intake in comparison to cage-reared birds. Beta-glucan supplementation also increased the retention of dry matter, calcium, and phosphorus as compared to the non-supplemented treatments. The authors attributed the success of β-glucan supplementation to its ability to modulate the immune system (Chae et al., 2006). This activity proved more beneficial to birds reared on litter rather than in cages due to the increased susceptibility to stress by litter reared birds (Chae et al., 2006). Thus it was concluded that an interaction exists between the impact of β-glucan on performance and the immune status of the animal (Chae et al., 2006; Cox et al., 2010a).

Beta-Glucan Supplementation During an Eimeria Challenge**.** Coccidiosis is one of the most prevalent and greatest economic impacting diseases associated with intensively reared poultry (Shirley et al., 2006; McDonald and Shirley, 2009; Cox et al., 2010b), with an estimated global impact in excess of $3.66 billion through vaccination and mitigation practices (Dalloul and Lillehoj, 2006; Shirley et al., 2006). Coccidiosis is a protozoal parasite of the gut, caused by host specific species (McDonald and Shirley, 2009). Broilers are host to seven *Eimeria* species, including: *E. tenella, E. maxima, E. acervulina, E. brunette, E. mitis, E. necatrix,* and *E. praecox* (McDonald and Shirley, 2009). These parasites are transmitted through consumption of oocysts from a host's fecal material (McDonald and Shirley, 2009). Upon entering the intestine, sporozoites begin to penetrate epithelial cells, disrupting the enterocyte layer and resulting in gross lesions along the intestinal wall (Brake et al., 1997; McDonald and Shirley, 2009). Asexual and sexual reproduction within the host enterocytes ultimately results in new generations of oocysts production to be shed through the feces (McDonald and Shirley, 2009). Spread of oocyst through a flock can occur within three to four weeks depending on the amount of oocyst shedding and consumption through the litter (Williams, 1994). Weight loss, listlessness, loss of appetite, diarrhea, and huddling are all observational signs of coccidiosis (McDonald and Shirley, 2009). Lesions along the intestinal lining will also result in reductions in nutrient absorption and overall performance of a host broiler (Brake et al., 1997).

Immunity to invasive *Eimeria* species involves T cells in a primary immune response (Rose and Hesketh, 1986) through IFN-γ (Rose et al., 1991). IFN-γ have been observed to decrease oocyst shedding and reduce weight loss during infection (Lillehoj and Choi, 1998). $CD8^+$ cells have also been attributed to *Eimeria* immunity during a secondary infection (Rose et al., 1992). The depletion of $CD8^+$ cells has been associated with an increase in oocyst shedding, implying the importance of these cells in preventing re-infection and spread of coccidiosis (McDonald and Shirley, 2009).

Control of coccidiosis had previously been mitigated through prophylactic chemotherapy with anti-coccidial drugs in broiler diets, until regulation and the ultimate ban on antibiotics occurred (Shirley et al., 2004). Because of the immune benefits offered by β-glucans, research has been conducted to evaluate the use of these products in the presence of an *Eimeria* challenge. In a study utilizing YBG at 0, 0.02, and 0.1%, Cox et al. (2010b) found no impact on performance of YBG to broilers challenged with *E. acervulina*, *E. maxima, and E. tenella*. However, significant reductions in lesion scores within the duodenum and jejunum were observed in broilers fed 0.1% YBG, exhibiting the immunoprotective properties of β-glucan against *E. acervulina* and *E. maxima*. Cox et al. (2010b) also observed enhancement of iNOS in challenged birds, exhibiting the capability of β-glucans to enhance the innate immune response. Overall, the immune response induced by β-glucan peaked at d 14 of the study, declining by termination at d 21. The authors suggested that β-glucans prime the immune system of broilers, providing anti-inflammatory effects. Beta-glucans can survive for extended periods of times within broilers due to the lack of endogenous β-glucanases (Miura et al., 2003). Thus, β-glucans are available to the immune system for extended periods of time (Soltanian et al., 2009), allowing for a priming of the immune system.

Shanmugasundaram et al. (2013) also evaluated the use of a commercially available YBG in *Eimeria* challenged broilers. In their report, the authors observed increases in BW and reductions in FCR with the supplementation of YBG at 0.2% 12 d post challenge. These benefits at a greater dosage reiterate that dosage level of β-glucan may factor in product effectiveness (Zhang et al., 2008; Vancaeneghem et al., 2000; Cox et al., 2010a). Additionally, fecal oocyst shedding was reduced with β-glucan supplementation 7 d post challenge. Inflammatory cytokine production post-coccidial infection was also increased with β-glucan supplementation, similar to the results reported by Cox et al. (2010b).

Beta-Glucan Supplementation Following a New Castle Disease Virus Vaccination. The Newcastle Disease Virus (NDV) is another disease that can prove very costly to the poultry industry if outbreak were to occur. NDV has been designated avian paramyxovirus 1, with outbreaks first reported in Indonesia and England in 1926 (Kranevald, 1926; Doyle, 1927; Seal et al., 2000). The virus has since spread worldwide, with entrance to the United States through the illegal importation of exotic birds (Fancis, 1970-71; Senne et al., 1983; Panigrahy et al., 1991; Bruning-Fann et al., 1992). NDV is spread through airborne transmission, with passage taking place either through ingestion or inhalation by broilers (Meulmanns, 1988). The disease can be divided into three pathotypes depending on the severity of the disease including: lentogenic isolates, which are mildly virulent; mesogenic isolates, which are of intermediate virulence; and velogenic isolates, which are highly virulent viruses that cause high mortality (Alexander, 1989 and1997; Seal et al., 2000). A 2003 outbreak of NDV in California resulted in the euthanasia and destruction of three million birds from 2,148 sites that included 22 commercial poultry farms (CIDRAP, 2003). The resulting cost of quarantine, destruction, and control of the outbreak was $160 million (CIDRAP, 2003).

The use of live and inactivated vaccines has been used in the prevention and control of NDV (Meulmanns, 1988). Vaccines induce antibody production of IgA, IgG, and IgM to high levels of vaccinated chicks (Russell and Ezeifka, 1995). $CD8^+$ and type 2 helper T cells are also noted to play a role in protection and immune response to NDV (Russell et al., 1997; Lessard et al., 1997). Because β-glucan can play a role in stimulating these immune responses, the use of β-glucan products in poultry vaccinated with NDV has been studied. In a study by An et al. (2008), YBG was fed to NDV vaccinated broilers at levels of 0, 0.025, and 0.1% for 35 d. The inclusion of YBG significantly increased d 35 BW of NDV vaccinated broilers. Additionally, NDV specific antibody titers were elevated through the supplementation of YBG at 0.1%. The cell mediated immune response was suggested by Cheng et al. (2004) to be improved by β-glucan through modulating macrophage activity. These results contradict those by Morales-Lopez et al. (2009), who found no benefits to performance or NDV specific antibody titers of a purified β-(1,3)-(1,6)-glucan when fed to NDV vaccinated broilers.

The inconsistencies indicated by β-glucan supplementation in non-challenged settings versus *Eimeria* challenge and presence of a NDV vaccine program has been attributed to utilizing different sources (Huff et al., 2006; Zhang et al., 2008; Soltanian et al., 2009; Cox et al., 2010b) and dosage levels (Vancaeneghem et al., 2000; Zhang et al., 2008; Soltanian et al., 2009; Cox and Dalloul, 2010) for products containing β-glucan. Because of this, other source alternatives should be researched, identifying proper dosage level to benefit performance and modulate the immune system.

Algae Derived Beta-Glucan. Historically, algae have been used by humans for nearly 2000 years, with the first known uses by the Chinese (Spolaore et al., 2006). Algal biotechnology has seen extensive growth in the last half century, producing 5000 tonnes of dry matter per year for a turnover of $1.25 billion per year (Pulz and Gross, 2004) The chemical composition of algae make it a unique product for multiple uses. Algae synthesize all amino acids, making the plant valuable to animal producers in terms of nutrition (Guil-Guerrero et al., 2004). Carbohydrates exist in the forms of starch, glucose, sugars, and other polysaccharides with a high level of digestibility (Becker, 2004; Spalore et al., 2006). Multiple algae species are utilized as food sources resulting in a wide range in value for protein, carbohydrate and lipid composition. Spalore et al. (2006) reports that general protein, carbohydrate, and lipid composition of algae range from 28-71%, 1-77%, and 1-34%, respectively, dry matter. Because of this, use of algae in livestock and poultry feeds has been explored.

Livestock and poultry feed applications account for 30% of global use of algae (Becker, 2004). Before algae are used as any nutritional source they must be analyzed for toxic compounds (Rebolloso Fuentes et al., 2001a,b). Considerations must be made for nucleic acid levels, with recommended feeding levels not to exceed 0.3 g of algae per kg of body weight consumed per day (Becker 1988, 2004). In a study conducted by Price et al. (2013), broilers and layers were fed post-extraction algal residue at levels of 0, 5, 10, 15, and 20%. Complete analysis of the algae was conducted prior to diet formulation, with crude protein and fat content analyzed at 20.2 and 1.56%, respectively. In a 35 d layer study, the group found no effect on feed consumption and egg production when feeding the algal residue to white leghorn hens, although yolk color was influenced when algal residue was fed at 10% of the diet for 14 days. An additional study found no adverse effects on performance parameters when algae were fed to broilers for 21 d. Because broiler skin and shank as well as yolk color of laying chickens is affected by diet, algae inclusion levels in poultry diets has been recommended not to exceed 5-10% of the total diet (Becker, 2004; Spolaore et al., 2004).

Algae contain a molecular structure similar to that of yeast cell walls, whose effects on immune status and performance of broilers was previously discussed. The most important structure to algae as an immunostimulator is β-(1,3)-glucan (Iwamoto, 2004), with health promoting effects on gastric ulcers, preventive action against atherosclerosis and hypercholesterolemia, and antitumor effects found in humans (Yamaguchi, 1997; Jong-Yuh and Mei-Fen, 2005). With inconsistencies in the literature on the effect of yeast derived β-glucan, investigations into alternative sources of β-glucan are necessary. A novel algal derived β-(1,3)-D-glucan product (Algamune ZPC™) has been developed as an alternative to yeast derived β-glucan products. The novel product is described as an algae meal, containing 45% linear β-(1, 3)-D-glucan, observed in Figure 2-2, by mass that is highly bioavailable without extraction or significant processing (Algal Scientific, 2013). Greater than 90% of the algal meal is β-(1,3)-D-glucan, noted to be the most immunologically active form (Algal Scientific, 2013).

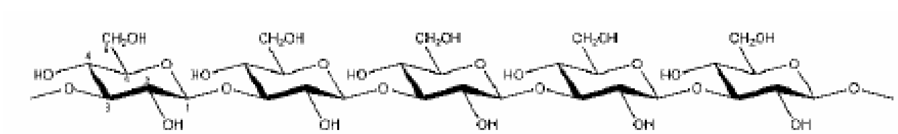

Figure 2-2. Linear, β-(1,3)-D-glucan structure of novel ABG. (Algal Scientific, 2013).

The purpose of this research was to evaluate the efficacy of the novel algal β-glucan (ABG) on broiler performance and immune status in a series of three experiments, including a 42 d growout in a non-challenge setting, a 20 d battery experiment during an *Eimeria* challenge setting, and following a Newcastle Disease Virus vaccination and boost.

Improvement of Nutrient Utilization Through the use of Feed Additives

Differences in polysaccharide physical properties are attributed to the way monomer units within the structure are linked (Moms, 1992). Although β-(1,3)-(1,6)-glucans derived from cell walls from multiple sources have proven to be beneficial, though inconsistent, to broiler immune status, a conformational change to β-(1,3)-(1,4)-glucans impact nutrient digestion and absorption of monogastric animals (Annison, 1993; Caprita et al., 2010). Beta-(1,3)-(1,4)-glucans are classified as non-starch polysaccharides (NSP), which are the major components of dietary fiber (Cummings et al., 1997) from plant material that is not hydrolyzed by endogenous enzymes but may be digested by microflora in the gut (Caprita, et al., 2010). NSP can be divided as either insoluble dietary fiber, which includes cellulose, hemicellulose, galactomannans, xylans, and xyloglucans; or water soluble dietary fiber, including pectins, arabinoglactans, arabinoxylans, and β-(1,3)-(1,4)-D-glucans (Caprita et al., 2010). The variety of NSP is based on varying degrees of water solubility, size, and structure of individual plant cell walls (Caprita et al., 2010), with water solubility playing an important role in the antinutritive effect of NSP (Annison, 1993).

Non-Starch Polysaccharide Structure. Water-soluble β-(1,3)-(1,4)-glucans and arabinoxylans are the major NSP of concern when feeding diets containing cereal grains to broilers (Caprita et al., 2010). Just as the β-(1,3)-(1,6)-glucans, β-(1,3)-(1,4)-glucans are linear chains composed of glucose containing β-(1,3)- and -(1,4)- glycosidic links; arabinoxylans are also composed of a long chain backbone containing β-(1,4) anhydro-D-xylopyranosyl attached to α-L-arabinofuranosyl residues at the 2- or 3-position

(Lineback and Rasper, 1988; Caprita et al., 2010) (Figure 2-3). Annison (1993) states that the greater the degree of branching of these structures, the higher the solubility and the greater the impact of the anti-nutritive effects of NSP.

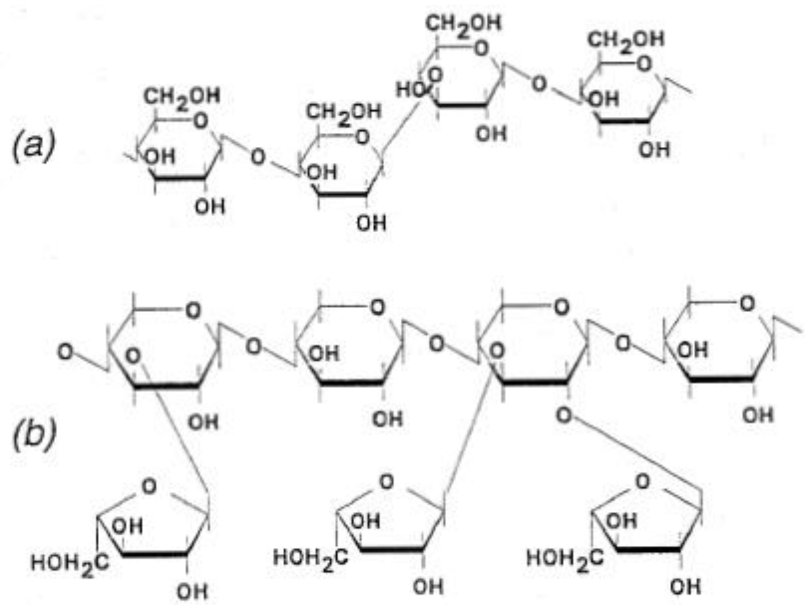

Figure 2-3. (a) β-(1,3)-(1,4)-glucan and (b) arabinoxylan structures of cereal grain cell walls (Annison, 1993).

Many cereal grains and grain products are available to broiler producers across the globe for use in diets. Grains chosen for diets are typically a reflection of the grains readily available at a reasonable price in the region (Bedford, 1995). Corn is the most abundantly grown cereal crop in the world (Cowieson, 2005), and is the most predominantly used grain in livestock feeding operations (Kiarie et al., 2014) in the

United States along with soybean meal (SBM). Corn is a popular choice due to its high starch content of greater than 600 g/kg (Weurding et al., 2001 a,b) and thus energy value to poultry of ~3344 kcal/kg (Cowieson, 2005). Corn is also low in NSP content, containing only 1g/kg soluble NSP (Choct, 1997) and a range of 4.7-10.7% insoluble NSP (Bach Knudsen, 2011; Kiarie et al., 2014). Dried distillers grains with solubles (DDGS) are a by-product of the ethanol industry from the fermentation of corn starch and other cereal grains, and is considered a rich source of crude protein, amino acids, phosphorus and other nutrients (Swiatkiewicz and Koreleski, 2008). Variability in the digestibility of the high gross energy from the high level of fat in DDGS is associated with NSP content of DDGS sources (Swiatkiewicz and Koreleski, 2008). DDGS contain 2-3 times the NSP content of corn (Cromwell et al., 1993; Belyea et al., 2004), with xylan being the predominant NSP (Liu et al., 2011) and an total NSP content of 250 g/kg (Swiatkiewicz and Koreleski, 2008). A considerable amount of soluble arabinose has also been detected within DDGS (Widyratne and Zijlstra, 2007). Thus use in diets will lead to an increase in NSP concentration in feed (Kiarie et al., 2014). Choct and Annison (1990) ranked cereal grains by total NSP content from low to high in the following order: rice, sorghum, corn, wheat, triticale, rye, and barley. NSP dry matter content accounts for 11.4% of the wheat kernel, with arabinoxylan being the predominant NSP located in the aleurone, testa, and pericarp layers (Caprita et al., 2010), representing a total of 50% of the total NSP content (Henry, 1984). NSP have been observed to be present at 61g/kg of wheat (Annison, 1993). In a study by Kiarie et al. (2014), wheat diets contained more than twice the amount of soluble NSP content than corn, with 7.25 and 5.35 times greater the concentration of soluble arabinose and xylose, respectively. High soluble NSP content is responsible for a reduction in dietary AME, and has been attributed to diets utilizing wheat at high levels (Annison, 1993).

***Anti-Nutritive Effects of NSP* – Viscosity.** Physiochemical properties of nutritional importance regarding NSP include hydration properties, viscosity, cation exchange capacity, and organic compound absorptive properties (Bach Knudsen, 2001). Water holding and binding capacity are influenced by NSP hydration properties (Bach Knudesen, 2001). Of utmost concern in diets containing high concentrations of NSP is the influence cereal grains play in feed and digestive viscosity (Annison, 1993). Viscosity influences associated with NSP are dependent on the molecular weight and size of the structure, the ion charged groups, surrounding structures, and the concentration of NSP (Smits and Annison, 1996). Viscosity is a function of both the concentration of soluble carbohydrate and, more importantly (Cowieson et al., 2005), the degree of polymerization of the carbohydrate (Izydorczyk and Biliaderis, 1992; Nilsson et al., 2000). It was suggested by Cowieson et al. (2005) that the viscosity of a solution can be high, even if the solution contains a low concentration of soluble NSP, if the NSP are of high molecular weight. NSP influence on increases in digesta viscosity (Choct et al., 2010) has been suggested to increase water consumption and thus increase fecal moisture of birds fed viscous grains (Francesch and Brufau, 2004). Because monogastrics cannot digest dietary NSP (Bedford, 1995; Meng et al., 2005), an increase in viscosity can lead to a decreased rate of digesta passage within the small intestine (Annison, 1993; Bedford, 1995; Bedford and Schulze, 1998; Choct et al., 2010) and a decrease in contact between digestive enzymes and target nutrient substrates (Annison, 1993; Bedford and Schulze, 1998), thus limiting exposure to brushy border enzymes for digestion, reducing absorption through the enterocytes (Johnson and Gee, 1981; Edwards et al., 1988; Bedford, 1995) and utilization by the bird. Bedford (1995) also indicated that an increase in digesta viscosity elevates microbial activity in the intestine. Diet viscosity has been suggested to impact younger birds to a greater degree than older birds due to the maturity of the GI tract in older birds and the ability to cope with soluble polysaccharides (Yasar and Forbes, 1999 and 2000).

Kiarie et al. (2014) compared the effects of corn and wheat based diets in a 42 d growout experiment. Diets were formulated to contain equivalent nutrient profiles. Upon analysis, wheat diets contained nearly twice the concentration of soluble NSP than corn. Corn diets contained 0.95 percentage points greater insoluble NSP as compared to wheat. This resulted in greater jejunal viscosity readings for wheat-based diets over corn. BW and feed intake were unaffected. Cereal grain source also impacted volatile fatty acid (VFA) profiles of broilers, with higher concentrations of cecal acetic and butyric acids than birds fed corn diets; however, broilers fed corn diets exhibited increases in cecal propionic, valeric, and iso-caleric acids as compared to wheat diets. Digestibility of fat was also influenced by cereal grains, with broilers fed wheat based diets exhibiting higher ileal digestibility of fat compared to corn diets. It was suggested by Slominski (2011) that measuring starch digestibility in the ileum better reflects the response of enzyme addition to broilers. Finally, retention of dry matter, phosphorus, and neutral and acid detergent fiber were greater in broilers fed corn-based diets.

Poultry feed is manufactured in high temperature environments. Diets are conditioned using high heat steam to increase pellet quality (Abdollahi et al., 2010), moderately improving bird performance (Cowieson et al., 2005). Feed is also subject to increases in heat during grinding of grains and raw ingredients and through heat pick up via rollers during pelleting. Moderate heating of broiler feed to 80-85°C has been attributed to benefits in performance to broilers fed wheat-based diets (Nissinen, 1994; Nir et al., 1995; Silversides and Bedford, 1999). Gelatinization of starches (Tovar et al., 1991), degradation of heat-labile anti-nutrients, and destruction of cell walls during moderate heat processing improves the availability of nutrients in the feed to broilers, improving performance (Pickford, 1992). However, pelleting wheat-based diets equal to or above 90°C has been observed to negatively impact broiler performance (Nissinen, 1994; Silversides and Bedford, 1999; Cowieson et al., 2005),with increases in conditioning temperature decreasing the ileal digestibility of starch and nitrogen (Abdollahi et al., 2010). Abdollahi et al. (2010) compared the effects of extreme heating in corn and wheat based diets. In the report, increasing conditioning temperature decreased BW and increased feed intake in broilers fed wheat-based diets; however, birds fed corn-based diets conditioned at 60 and 90°C had higher BW and feed intake compared to those fed diets conditioned at 75°C. Starch digestibility in the corn-based diet was not impacted by conditioning temperature.

Extreme heating inactivates endogenous enzymes and vitamins, reduces the availability of protein and starch, and partially causes Malliard complexing of amino acids (Pickford, 1992; Batterham et al., 1993 and 1994; Brown, 1996; Silversides and

Bedford, 1999), thus impacting performance. Increases in feed viscosity have been observed in mash feed that is then pelleted (Cowieson et al., 2010). In a report by Cowieson et al. (2005), an increase in diet viscosity was observed when diets were pelleted above 80°C. It was suggested that this was a result of an increased release of encapsulated NSP, although the group observed a decrease in soluble xylan concentration with increasing pelleting temperatures. Increases in feed viscosity also decreases water absorption which in turn increases water losses through fecal material (Bedford, 1995; Francesch and Brufau, 2004).

Anti-nutritive effects of NSP – Encapsulation of nutrients. As mentioned previously, cereal grain seed coats are comprised of high concentrations of NSP. Because broilers do not possess the endogenous enzymes to hydrolyze this cell wall (Meng et al., 2005), arabinoxylan directly disrupt and inhibit digestion of starch, fat, and protein (Choct and Annison, 1990, 1992a; Caprita et al., 2010; Choct et al., 2010) through encapsulation of nutrients (Annison, 1993). The reductions in digestibility of nutrients by NSP depend on the source, composition, and inclusion level in the diet (Murray et al., 1977; Friere et al., 2000), resulting in reductions in dietary AME values for diets containing large proportions of grains high in NSP concentration (Annison, 1993). Reductions in AME results in a decrease in feed utilization, increasing feed intake (Annison, 1993) and overall feed cost of the producer.

Xylanase as a Feed Additive to Minimize Anti-Nutritive Effects of NSP. The use of exogenous enzymes, such as endo-β-(1,4)-xylanases and β-(1,3)-(1,4)-glucanases, in broiler diets has occurred commercially for over two decades (Bedford, 2000) to reduce the molecular size and impact of their respective substrates (Bedford, 1995). Bedford (2000) stated that enzymes are utilized in poultry diets to increase the feeding value of raw materials; reduce the variation in nutrient quality of ingredients, improving flock uniformity, improving uniformity from flock to flock, and reducing the incidence of wet litter. Two main modes of action have been proposed for the use of exogenous carbohydrases in broiler diets: the viscosity theory and the 'cage effect' (Bedford, 2002; Cowieson et al., 2006). The viscosity theory encompasses all benefits associated with the use of supplemental xylanase on viscosity and the additional advantage of the enzyme on the digestibility of nutrients within the diet (Cowieson et al., 2006). The 'cage effect' theory is associated with the release of previously encapsulated nutrients within grain cell walls by carbohydrase enzymes (Bedford, 2002). The result of supplementation is an increase in the rate of nutrient digestibility, regardless of mode of action (Bedford, 2000).

Use of Xylanase to Combat Anti-Nutritive Effects of NSP in Wheat Diets. The first theory proposed in carbohydrase mode of action is the viscosity theory. Bedford (1995) stated that the reduction in molecular size by carbohydrases on their substrates reduces viscosity. Because chain link is a function of viscosity, carbohydrases prove beneficial in reducing digesta viscosity through degradation of NSP linkages (Bedford, 1995), cleaving the xylose backbone of arabinoxylan (Meng et al., 2005) and eliminating the gel-forming capacity of NSP (Chesson, 1993). Viscosity impacts on rate of digestion are also offset, allowing for a more rapid rate of digestion (Bedford, 1995). A visualization of the mode of action of enzymes in the breakdown of barley cell walls was made by Lee et al. (1999), (Figure 2-4). It is important to note that it is unlikely that carbohydrases reduce their substrates to their constituent monosaccharides since few catalytic reactions are necessary to reduce the molecular size and viscosity of the substrate (Bedford, 1995).

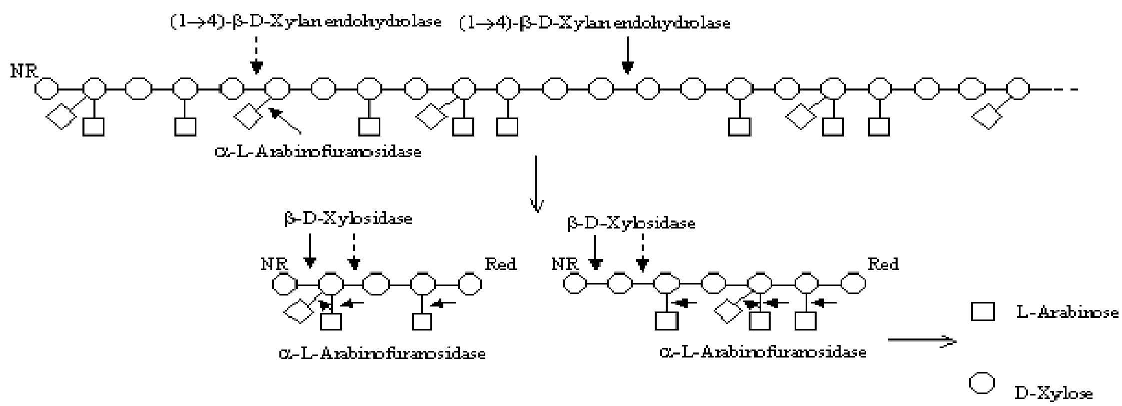

Figure 2-4. Arabinoxylan structural model with proposed sites of action of major arabinoxylan hydrolyzing enzymes and sequential stages of complete depolymerisation (Lee et al., 1999).

The use of supplemental xylanase to reduce diet viscosity and thus benefit growth performance has been studied. Choct et al. (1999) reviewed the use of xylanase at 2500 U/g in a diet containing 20 g/kg soluble NSP in low-AME wheat-based diets. Significant reductions in gut viscosity were detected with xylanase supplementation in each section of the small intestine, with the greatest impact on ileal viscosity. Ileal starch digestibility was improved to 98% in broilers fed supplemental xylanase. It was suggested that the increase in starch digestibility was a result of the reductions in digesta viscosity. Enzyme supplementation also significantly reduced the variability in starch digestibility amongst treated birds. This is of significant importance to the broiler industry as it leads to more consistent and uniform performance of flocks. These benefits to the gut allowed for reductions in FCR for xylanase supplemented broilers. AME was also significantly improved in the xylanase supplemented diet as compared to the control diet.

The reduction in diet viscosity by enzyme supplementation is also suggested to increase digesta passage and absorption rate (Choct et al., 1999). Absorption of digestive products must occur through the intestinal lumen to the enterocyte (Bedford, 1995). The unobstructed diffusion of enzymes, substrates, and products is essential for rapid digestion (Bedford, 1995) and overall nutrient utilization. The reduction in viscous NSP substrate and increase in digesta passage is suggested to reduce fermentative organism proliferation, restoring enzymatic digestion of starch and protein in the small intestine to normal (Choct et al., 1999). This impact was observed by Choct et al. (1999) through the reductions of VFA production within the ileum with xylanase supplementation as compared to the control diet. Subsequently, ceca VFA profiles were enhanced with the supplementation of xylanase within the diet. Increased microbial activity in the gut is supported by increases in viscosity, with a decrease in oxygen tension and providing a relatively beneficial environment where fermentative microflora can proliferate (Wagner and Thomas, 1978). Increased microflora activity and fermentation of nutrients reduces access to nutrients for digestion and absorption.

Apparent Metabolizable Energy accounts for energy loss in the excreta of broilers and thus is an expression of energy utilization by the broiler. In a report by Nian et al. (2011b), the addition of xylanase at 4000 U/kg to a reduced ME wheat-based diet increased diet AME by 4.2%. The low-AME phenomenon of wheat from a variety of sources has been documented by Annison and Choct (1991) due to the variable NSP content of wheat. The energy releasing action of xylanase through the release of entrapped starch provides an opportunity to overcome this reduction in diets. Choct et al. (2004) evaluated the use of xylanase in low- and normal-ME wheat. The addition of xylanase improved the AME value of low-ME wheat during the study. Significant reductions in ileal viscosity were also only observed in xylanase supplemented low-ME wheat diets. Xylanase supplementation also improved BW and FCR in the report by Choct et al. (2004), though regardless of wheat type. Increases in ME value in wheat diets with the supplementation of carbohydrases has also been reported Brufau and Francesch, 1991 and Meng et al., 2005.

Improvements to nutrient utilization and digestion with the use of xylanase in wheat-diets lead to improvements in performance parameters. In the Nian et al. (2011b) study, numeric improvements to energy digestibility in the xylanase supplemented diet correlated into a significant improvement in FCR. These results were replicated by Cowieson and Massey O'Neill (2013), in which supplementation of 160000 U/g of xylanase reduced 49 d cumulative FCR by 6.7%. These benefits were observed in coordination with increases in d 28 and 42 ileal digestible energy levels by 83 and 214 kcals. Wang et al. (2005) also reported a positive linear increase in BW and reduction in

FCR in wheat-based diets supplemented with enzyme at 0, 200, 400, 600, 800, and 1000 mg/kg. The linear improvements to performance parameters indicate increased dosage responses to enzymes.

Further effects of NSP on GI tract size have also been investigated. Brenes et al. (1993) attributes large changes in digestive organ weights and sizes to the presence of viscous carbohydrates. The increase in size of the pancreas and digestive tract may be an adaptive response to the increased need for digestive enzymes (Brenes et al., 1993). Within their report, the authors detail the use of carbohydrase enzymes in wheat- and barley-based diets. Enzyme supplementation reduced the relative length of the duodenum, jejunum, and ileum as well as the relative weights of the proventriculus, pancreas, liver, duodenum, jejunum, ileum, and colon in the barley-based diet. Relative organ weights were unaffected by enzyme supplementation in the wheat based diet. However, Wang et al. (2005) reported a negative linear relationship between increasing enzyme supplementation and the effects on 21 and 42 d broiler liver and pancreas weights and intestinal segment length. The reduction in organ weight is thus suggested to have a direct economic impact, with WOG yields of carcasses increasing due to the reduction in organ weights (Brenes et al., 1993).

High Heat Feed Processing. The benefits of moderate heat treatment of broiler diets on broiler performance were discussed previously. Although moderate heating was observed to be beneficial to broiler performance, more extreme heating has proven to be detrimental. Enzymes utilized in diets must also be able to survive the range of heat exposure during feed processing as well within the gut, although some loss is inevitable

(Chesson 1993). To accomplish this, enzyme products are produced with a protective coating that can withstand extreme heat to prevent major losses in activity through heat processing (Bedford and Cowieson, 2009; Graham and Bedford, 2007; Amerah et al., 2011) and the internal temperature of broilers (Bedford, 2008).

To test the effects of xylanase in diets conditioned at extreme heat, Silversides and Bedford (1999) included xylanase at 2860 U/kg in wheat-based diets manufactured at 70, 80, 90, and 95°C. Feed was conditioned for 30 seconds at these temperatures to achieve desired temperature. Enzyme supplementation reduced viscosity at all temperatures. It has been suggested by Amerah et al. (2011) that enzyme response to intestinal viscosity are more pronounced in feeds manufactured at high temperatures. Processing feed over 85°C proved detrimental to broiler performance, regardless of enzyme supplementation. Interactions between enzyme, temperature, and processing time were observed in two way (BW) and three-way (BW, FCR) interactions, with the most desirable benefits to performance being determined between 80 and 85°C. However, Cowieson et al. (2005) concluded that wheat-based diets could be pelleted at temperatures as high as 90°C with no detrimental effects to performance with the inclusion of xylanase at 2000 U/kg.

Wheat vs. Corn. Because diet grain inclusion and diet composition vary by region and availability, it is important to compare the use of xylanase in all types of diets. Xylanase inclusion in wheat diets has been thoroughly studied as indicated above. Kiarie et al. (2014) compared the use of xylanase in wheat- and corn-based diets to compare effects on performance, nutrient utilization, and digesta characteristics. Diets were formulated using corn and wheat as the main cereal grains, with xylanase added to one dietary treatment of each grain source at 1250 U/kg in a 42 and 21 d experiment. The authors observed no interaction between diet and xylanase inclusion on performance. Inclusion of xylanase increased BW and decreased FCR in treated birds as compared to non-supplemented broilers. An interaction was observed between diet type and xylanase inclusion on jejunal viscosity, with xylanase inclusion decreasing both diet types to similar levels. No interaction between diet type and xylanase was observed on cecal VFA profiles, apparent ileal digestibility of nutrients, and AME_n. However, broilers fed xylanase were observed to have increases in these parameters as compared to non-supplemented broilers. These data suggested that xylanase improve growth performance and AME_n of broilers regardless of diet type.

Energy Reduced Diets. Starch is one of the most important nutrients within grains, accounting for 60-70% of the content in terms of grain weight (Annison, 1990). Soluble NSP's, mainly arabinoxylan (Henry, 1985), encrust the endosperm cell wall of cereal grains, entrapping starch within the grain and preventing access to endogenous enzymes within monogastrics (Bedford, 1995). Although low in NSP, an estimated 400-450 kcal of energy per kg of diet goes undigested by broilers in corn-SBM diets (Cowieson, 2010) representing a potential energy source for growth. The loss of energy to undigested fat, starch, and protein presents an opportunity to use exogenous enzymes to increase energy availability to birds (Cowieson, 2010). Carbohydrases can be beneficial in the release of entrapped starch and protein from the cell walls of grains, allowing for greater nutrient access when fed in broiler diets. Physical destruction of grain cell walls through grinding during feed processing opens access to these nutrients, but alone does not solve the issues associated with NSP's (Bedford, 1995). Disruption, rather than complete degradation, to endosperm cell walls requires enzymatic cleavage of fewer NSP linkages, allowing digestive enzymes access to previously entrapped nutrients (Chesson, 1993). The use of supplemental enzymes moves the site of digestion and absorption of starch and protein further down the GI, where Bedford (2000) states broilers have a competitive edge over its resident microflora, with a highly digestible diet being digested and absorbed prior to the establishment of an environment favorable to bacterial growth.

Cowieson and Masey O'Neill (2013) compared the effects of xylanase supplementation at 16000 BXU/kg in a 59 kcal/kg energy reduced wheat-based diet to a standard wheat diet. Xylanase supplementation improved BW and FCR at d 14, 28, and cumulatively on d 49 to levels of similar significance and numerically improved compared to the industry standard diet. Xylanase supplementation also increased the ileal digestibility of energy on d 49 in the energy reduced diet to levels greater than the industry standard diet. Because of these findings, it was concluded that producers could reduce the energy value in diets with no impact to performance through the use of supplemental xylanase.

The relative low level of NSP in corn does not pose a serious problem to digesta viscosity (Slominksi, 2011). Although starch is well digested by broilers fed corn-based diets, it is not completely digested (Classen, 1996; Slominski, 2011), with ileal digestibility rarely exceeding 85% (Noy and Sklan, 1994). As previously mentioned, the release of previously bound starch and increases in energy digestibility has been attributed to the use of xylanase in wheat-based diets. The use of supplemental xylanase in corn-based diets could provide benefits to energy digestion through the small fractions of NSP within the diet (Slominski, 2011). Thus an energy value can be assigned to xylanase when included in diets to contribute to the overall energy value (Masey O'Neill et al., 2012). To test this, energy can be removed from the diet through the removal of supplemental fat and replacement with corn, subsequently lowering the feed cost through the reduction of a costly energy source to the diet. However, it is important to note that it is unrealistic to expect to achieve 100% ileal digestibility of nutrients even with these enzymes (Cowieson et al., 2010).

Removing dietary energy from corn-based diets has resulted in impacts to performance through reductions in BW (Singh et al., 2012) and ileal digestibility of nutrients (Cowieson et al., 2010), and increases in FCR (Cowieson et al., 2010; Masey O'Neill et al., 2012; Singh et al., 2012). Masey O'Neill et al. (2012) stated that a review of published literature indicates a linear increase in feed intake when dietary energy is decreased. The use of supplemental xylanase in diets has been investigated to counter these effects. Coweison et al. (2010) investigate the use of xylanase inclusion at 16000 BXU/kg in a corn-based diet with an energy reduction of 110 kcal/kg over 42 d. Supplementation of xylanase in the energy reduced diet was able to improve FCR 4-5 points in broilers to similar levels of those fed a standard energy diet. An increase in ileal digestibility of 31 and 91 kcal/kg was observed with the addition of xylanase in the energy reduced starter and finisher feeds, respectively, significantly greater than the unsupplemented diet. However, digestibility of energy was not able to return to levels similar to the positive control. The benefits to FCR are supported by Masey O'Neill et al. (2012), in which supplementation of xylanase at 16000 U/g in energy reduced diets significantly improved FCR cumulatively at d 35 and 42. The authors suggested a dramatic development in the benefit of xylanase to FCR occurs over the life of the bird, because although no early effects to FCR were observed, significant decreases in FCR in xylanase supplemented broilers was observed cumulatively at d 35 and 42. In contrast, Williams et al. (2014) indicated improvements to d 15 BW and FCR in xylanase supplemented birds in diets with reduced energy levels of 66 and 132 kcal/kg. The early improvements to FCR were observed cumulatively through the termination of the project on d 45. However, variation still exists in literature, with Nian et al. (2011a) and Singh et al. (2012) both reporting no benefits to performance with the use of xylanase in energy reduced diets, although Nian et al. (2011a) did report a 2% increase in ileal digestibility of energy.

Because profits are made off of end products of broilers and not their final BW, the processing parameters of these birds should be quantified. In the report by Williams et al. (2014), supplementation of xylanase in the energy reduced diet decreased fat pad weights and fat pad yield. The authors suggested that increases in xylanase inclusion may positively increase carcass yield. Brenese et al. (1993) suggested that increases in carcass yields should be observed due to the reductions in organ weights caused by xylanase supplementation. In a similar fashion, Coppedge et al. (2012) observed improvements to breast meat yield in broilers supplemented with a cocktail enzyme product, with the main enzyme being xylanase. These findings suggest that it is not only important to investigate the economic impact of enzymes on feed cost and performance efficiency, but also to the enhancements they may provide to broiler end-products.

The use of supplemental xylanase in both corn- and wheat-based diets to improve feed efficiency and performance is a global practice. The use of feed additives save the global animal market an estimated $3-5 billion a year, with average feed cost savings of $10-12 per metric tonne (Coweison et al., 2010). The variability in xylanase action and response by broilers has been suggested by Choct et al. (2004) to be a result of differing efficacy in degrading arabinoxylan due to their substrate affinity. It is also important to identify the proper dosage level of various products containing xylanase in the specific diet it is being used in to quantify their effect on performance. The purpose of this research was to identify the dosage response of an experimental xylanase on broiler performance, energy digestibility, and digestive parameters in diets containing wheat and DDGS and in a corn-SBM based diet.

CHAPTER III

EVALUATION OF A NOVEL ALGAL BETA-GLUCAN ON BROILER GROWTH PERFORMANCE AND IMMUNE RESPONSE

Introduction

The demand for limited and reduced use of antibiotics in poultry production has caused an increase in use of alternative supplements to benefit immune function and growth performance. The rise in antibiotic-resistant bacteria has caused a need for new forms of disease prevention (Cox and Dalloul, 2010). One possible alternative that has been extensively investigated is the inclusion of β-(1,3)-glucans in poultry diets. Beta-(1,3)-(1,6)-glucans are a structural component of the cell wall of many bacteria, fungi, yeasts, and algea (Jorgensen and Robertsen, 1995). Variations in β-glucan structure, molecular weight, degree of branching, length of the main chain, and intermolecular association from varying sources results in different physiological functions (Wagner et al., 1988; Jamas et al., 1991; Bohn and BeMiller, 1995; Kulicke et al., 1997; Pins et al., 2005a and b; Yoshitomi et al., 2005; Leung et al., 2006; Volman et al., 2008).

Beta-glucans are classed as biological response modifiers due to their ability to stimulate the immune system (Cox and Dalloul, 2010). Immune modulation has been attributed to β-glucan due to its highly branched structure and insolubility (Vetvicka and Vetvickova, 2007; Zhang et al., 2008; Cox et al., 2010b) and their ability to activate macrophage (Sakurai et al., 1992; Guo et al., 2003) and other immune regulatory cells. In particular, yeast derived β-glucans have been suggested to modulate both specific and non-specific immune responses in animals (Chae et al., 2006). Nearly 85% of the cell wall of yeast is composed of branched β-(1,3)-glucan of high molecular weight, with an additional 3% β-(1,6)-glucosidic interchain linkages (Manners et al., 1973) and are considered to be highly effective as immune regulators due to their highly branched structure (Vetvicka and Vetvickova, 2007; Harada and Ohno, 2008). Length of the main chain, and thus the activity of β-glucans, is also contributed to the variety of types and sources of β-glucan available (Wagner et al., 1988; Jamas et al., 1991; Bohn and BeMiller, 1995; Yoshitomi et al., 2005; Leung et al., 2006).

Inconsistencies have been reported in literature which indicate that β-glucans can improve performance through increased body weights (An et al., 2008; Rathgeber et al., 2008) and reduced FCR (An et al., 2008; Rathgeber et al., 2008; Morales-Lopez et al., 2009); decrease BW of non-challenged birds (Huff et al., 2006); or have no effect on broiler performance (Cox et al, 2010a; Chae et al., 2006; Rathgeber et al., 2008; Zhang et al., 2012) in challenge and non-challenge settings. It is suggested that the immune status of the animal may play a role in performance response by broilers fed β-glucan products (Chae et al., 2006; Cox et al., 2010a).

Numerous investigators have detailed the effects of yeast cell wall β-glucans and their effects on broiler performance and as an immune modulator with comparisons to antibiotic growth promoters as an alternative form of disease prevention (Chae et al., 2005; Rathgeber et al., 2008; Morales-Lopez et al., 2009; Cho et al., 2013). Coccidiosis and Newcastle Disease are diseases of interest in poultry production. *Eimeria* species are known to invade the intestinal tract of animals, disrupting digestion, impeding absorption, and causing performance losses to the host. Evidence of an *Eimeria* infection is left in the intestinal tract in the form of lesions (Brake et al., 1997). The use of a vaccination program to combat *Eimeria* was reported by Lee et al. (2011) to impact broiler performance through reductions in BW and increases in FCR. These observations were most important at the early stages of life between d 13 and 17 (Lee et al., 2011). Yeast β-glucans have been observed to improve immune function and performance in broilers (Zhang et al., 2012). Beta-glucans have been shown to improve immune function in *Eimeria* challenged broilers through decreased fecal oocyst counts (Shanmugasundaram et al., 2013) and reduced intestinal lesions and severity (Cox et al., 2010b). The use of live-virus vaccines to combat Newcastle Disease is known to increase IgA, IgG, and IgM antibodies in d old broilers (Meulmanns, 1988; Russell and Ezeifka, 1995). Analysis of Newcastle specific antibodies through antibody titer measurements is a proven diagnostic method to determine the immune status of broilers vaccinated with Newcastle Disease vaccines (Alexander, 1997). An et al. (2008) reported an increase in Newcastle disease specific antibody titers in Newcastle vaccinated broilers fed β-glucans in the diet. An increase in size of primary and secondary lymphoid organs has also been associated with β-glucan supplementation in feed, suggesting a route for their immunological effects (Guo et al., 2003; and Zhang et al., 2008).

With the numerous reports of inconsistencies in results with yeast derived β-glucan, further investigation is needed on the effectiveness of alternative sources of β-glucan. A review of literature indicated no previous experiments evaluating an algal derived β-glucan product on poultry performance and immunological effects. The objective of this series of experiments was to investigate the effects of an algal β-(1,3)-glucan (ABG) product (Algamune ZPC™, Algal Scientific Corporation) on broiler performance, oocyst output following an *Eimeria* challenge, and antibody titers following Newcastle vaccination and boost in a series of three experiments. Algamune ZPC™ is a zinc metal polysaccharide product that contains 45% β-(1,3)-glucan derived from a whole algae meal for use in animal feeds to supplement immune function. 90% of the β-glucan within Algamune ZPC™ exist as (1,3) linear chain linkages (Algal Scientific, 2013).

Materials and Methods

Experimental Design. A series of three experiments were conducted to evaluate the effect of ABG on broiler growth performance during a 42 d growout, oocyst output and performance during an *Eimeria* challenge, and Newcastle Disease specific antibody titers following a Newcasle Disease vaccine and boost. One large basal diet was manufactured for each dietary phase and experiment, and ABG was included prior to pelleting at levels of 0, 100, 250, and 750 g/MT. All experiments were conducted in accordance with an approved animal use protocol (IACUC). Broilers were provided age appropriate heating and given access to feed and water *ad libitum*. Broilers were fed an industry type dietary program (Table 3-1).

Experiment 1. A 42 d growout experiment was conducted to evaluate the effects of dietary inclusion of ABG on broiler growth performance. On d of hatch, 1400 Cobb

Table 3-1. Dietary formulation and calculated nutrient content of diets, based on percentages, for male broilers fed Algamune ZPC[1] at increasing levels.

Ingredient	Starter	Grower	Finisher
Corn	58.19	63.36	68.47
Dehulled Soybean Meal (48%)	34.57	29.62	24.69
DL-Methionine (99%)	0.23	0.20	0.16
Lysine HCL	0.16	0.14	0.12
Fat, A/V Blend	2.90	2.90	2.92
Limestone	1.57	1.58	1.56
Monocalcium Phosphate	1.56	1.40	1.29
Sodium Chloride	0.51	0.49	0.49
Vitamin Premix[2]	0.25	0.25	0.25
Trace Minerals[3]	0.05	0.05	0.05
Calculated Nutrient Content			
Protein	22.00	20.00	18.00
Lysine	1.30	1.15	1.00
Methionine	0.56	0.51	0.44
TSAA	0.92	0.84	0.75
Threonine	0.82	0.75	0.67
Calcium	0.95	0.92	0.88
Total Phosphorus	0.70	0.66	0.63
Available Phosphorus	0.45	0.41	0.38
Sodium	0.22	0.21	0.21
Metabolizable Energy (kcal/kg)	3050	3100	3150

[1]Algamune ZPC is a zinc metal polysaccharide complex that contains beta-1,3-glucan from algae as part of the complex. This product is intended to provide a nutritional form of zinc metal that provides additional bioavailability when used with other nutritional forms of zinc. This product meets the AAFCO definition of metal polysaccharide. (Algal Scientific, Plymouth, MI, USA)

[2] Vitamin premix added at this rate yields 11,023 IU vitamin A, 3,858 IU vitamin D_3, 46 IU vitamin E, 0.0165 mg B_{12}, 5.845 mg riboflavin, 45.93 mg niacin, 20.21 mg d-pantothenic acid, 477.67 mg choline, 1.47 mg menadione, 1.75 mg folic acid, 7.17 mg pyroxidine, 2.94 mg thiamine, 0.55 mg biotin per kg diet. The carrier is ground rice hulls.

[3] Trace mineral premix added at this rate yields 149.6 mg manganese, 125.1 mg zinc, 16.5 mg iron, 1.7 mg copper, 1.05 mg iodine, 0.25 mg selenium, a minimum of 6.27 mg calcium, and a maximum of 8.69 mg calcium per kg of diet. The carrier is calcium carbonate and the premix contains less than 1% mineral oil.

500 males were weighed and randomly allotted to floor pens and dietary treatments based on body weight. Thirty-five broilers were placed per replicate pen, with 10 replicate pens per treatment for a total of 40 replicate pens. Stocking density was set to 1.00 sq. ft./bird. Pens contained recycled litter top dressed with fresh pine shavings. All broilers were vaccinated with a live oocyst vaccine upon arrival to the experimental location. The dietary program consisted of a starter (d 1-14), grower (d 14-28), and finisher (d 28-42). Broilers and feed were weighed at the end of each dietary phase on d 14, 28, and 42 for the calculation of average body weights and feed conversion ratio. On d 42, 15 birds per dietary treatment were randomly selected, weighed, and euthanized. Birds were necropsied, and the liver, spleen, and kidneys were removed and weighed to determine relative organ weight.

Experiment 2. Day old male broilers from the same hatch as males in Experiment 1 were placed in battery brooders for a 20 d experiment evaluating the effects of ABG during an *Eimeria* vaccine challenge setting. Starter feed manufactured for Experiment 1 was used for the duration of the experiment, with two separate treatment groups being fed the control diet. Ten replicate pens were used per dietary treatment with seven male broilers per replicate, for a total of 350 male broilers used in the experiment. On d 10, all broilers fed ABG and one control treatment were challenged with a 100X dose of vaccine strain *Eimeria* oocysts (Coccivac®-B) containing *Eimeria acervulina*, *E. maxima*, *E. mivati*, and *E. tenella*; the remaining control group was not vaccinated as a non-challenged control group. Body weights and feed weights were recorded on d 10 (d of challenge), 17 (6 d post challenge), and 20 (termination of the experiment) for the determination of performance effects of ABG during an *Eimeria* challenge. On d 17, six days post-challenge, three broilers per replicate pen were euthanized and necropsied, with intestines removed for assessment of lesion development associated with the *Eimeria* vaccine challenge. Intestines were removed for evaluation of gross lesions associated with the challenge and given a value of 0 through 4, wherein 0 is normal and 1, 2, 3, or 4 indicate increasing severity of infection (Johnson and Reid, 1970). Sampling sites included the ascending and descending loops of the duodenum (upper intestine), five cm anterior and posterior of Meckel's diverticulum (middle intestine), and both ceca (lower intestine). Fecal material was collected from each replicate pen 6, 7, 8, and 9 d post challenge to quantify oocyst shedding associated with challenge using the method outlined by Oden et al. (2012). Fresh fecal droppings (minimum of 8 individual droppings per pen) on manure pans were collected and pooled for examination and quantification of oocysts present per gram of fecal contents. Prior to analysis, each fecal sample was homogenized, weighed, and diluted at a 3:1 ratio of water to fecal matter. Following agitation, fecal suspension was extracted and loaded into a hemacytometer to be observed microscopically for oocyst presence. A standard light microscope with 10× eye-piece objective and a 20× objective (200× magnification) was used to quantify non-sporulated oocysts present in each sample for oocyst per gram of feces (OPG) calculations.

Experiment 3. A third experiment was conducted to determine the effects of ABG on performance and Newcastle Virus specific antibody titers of broilers following a Newcastle/Bronchitis vaccination program. Experimental treatments remained consistent to the previous experiments. Five male broilers were placed in each replicate pen, with 6 replicate pens per treatment for a total of 120 Cobb 500 males used in the experiment. A starter feed consistent with the first two experiments was fed through 18 d. A grower feed was manufactured without ABG inclusion and was fed from d 18 to 25. On d of hatch, each chick received 100 µl of Newcastle/Bronchitis vaccine diluted to the equivalent of a full dose; 50 µl was administered to each bird through intranasal and 50 µl intraocular. Broilers received a vaccine boost at d 18 consistent in dosage and administered through the same route as the initial vaccination. On d 25 (7 days post boost), blood was obtained from all broilers. Samples were allowed to clot overnight and centrifuged to obtain the serum. Serum antibody titer levels were determined via ELISA using a commercially available kit (IDEXX Newcastle Disease Virus Antibody Test Kit). Body weights and feed weights were measured on d 7, 18, and 25 for the determination of performance effects of ABG during a Newcastle vaccination program on the basis of body weight, average daily feed consumption, and mortality corrected feed conversion.

Statistical Analysis

Relative organ weights from Experiment 1, lesion scores and oocyst output from Experiment 2, antibody titer levels and feed consumption from Experiment 3, and body weight and mortality corrected feed conversion ratio from all experiments were analyzed *via* Analysis of Variance (ANOVA) using the General Linear Model Procedure using SPSS V 18.0. Main effect means were deemed significantly different at $p<0.05$ and separated using Duncan's Multiple Range Test. The experimental unit for each parameter evaluated was replicate pen.

Results

Experiment 1. On d 14, inclusion of ABG at 750 g/MT in the control diet significantly increased ($p<0.05$) body weight (BW) as compared to the control diet (Table 3-2). Inclusion of ABG at 100 g/MT in the control diet negatively influenced BW ($p<0.05$) as compared to the control diet on d 14 and 28. On d 28, inclusion of ABG at 250 and 750 g/MT did not impact BW as compared to the control diet. At the conclusion of the experiment on d 42, all experimental treatments yielded similar body weights.

With regards to mortality corrected feed conversion ratio (FCR), inclusion of ABG at 100 g/MT in the control diet significantly increased ($p<0.05$) FCR during the starter phase as compared to the control diet. The inclusion of ABG at 250 g/MT resulted in a lower FCR during the starter phase as compared to the control diet (Table 2). During the grower phase, the inclusion of ABG in the control diet had no impact on FCR as compared to the control diet. The inclusion of ABG at 100 g/MT in the control diet significantly reduced FCR as compared to the inclusion of ABG at 250 g/MT during the grower phase. Inclusion of ABG in the control diet had no impact on FCR during the finisher phase as compared to the control diet. Cumulative FCR was not impacted by ABG as compared to the control diet from d 1-28 and at the conclusion of the experiment from d 1-42.

Table 3-2. Average BW, dietary phase mortality corrected FCR, and cumulative mortality corrected FCR of male broilers fed increasing levels of ABG in a non-challenge setting (Experiment 1).

Treatment	Body Weights			Dietary Phase FCR			Cumulative FCR	
	Day 14 (g)	Day 28 (kg)	Day 42 (kg)	Starter	Grower	Finisher	Day 1-28	Day 1-42
Control (Cont.)	383.5[b]	1.524[a]	3.049	1.336[b]	1.534[ab]	1.663	1.453	1.558
Cont. + 100 g/MT ABG	341.2[c]	1.433[b]	2.904	1.395[a]	1.489[b]	1.642	1.452	1.543
Cont. + 250 g/MT ABG	389.7[ab]	1.539[a]	3.062	1.290[c]	1.591[a]	1.668	1.472	1.563
Cont. + 750 g/MT ABG	395.4[a]	1.513[a]	2.974	1.321[b]	1.538[ab]	1.697	1.457	1.568

[a-c]Means in columns differ at $p<0.05$

Inclusion of ABG in the control diet had no impact on organ weight or relative weight of liver, spleen, and kidney weights as compared to the control diet at the conclusion of the trial (Table 3-3).

Experiment 2. On d 10 (day of challenge), inclusion of ABG at 250 g/MT increased ($p<0.05$) BW as compared to control fed broilers while the 100 and 750 g/MT had similar BW to the control diet (Table 3-4). On d 17 and 20 (7 and 10 days post-challenge, respectively) inclusion of ABG at 250 and 750 g/MT increased ($p<0.05$) BW as compared to the non-challenged control broilers. *Eimeria* challenge did not impact body weight gain during challenge in control fed broilers, as non-challenged and challenged broilers had similar weight gain. Broilers fed 100 g/MT of ABG had a reduced ($p<0.05$) rate of weight gain as compared to control groups from d 10-17 and d

Table 3-3. Organ and relative organ weights of broilers fed increasing levels of ABG in a non-challenge setting (Experiment 1).

Treatment	Weights (g)				Relative Organ Weight (%)		
	Body WT	Liver	Spleen	Kidney	Liver	Spleen	Kidney
Control (Cont.)	2900.0	71.9	3.4	13.8	2.48	0.12	0.47
Cont. + 100 g/MT ABG	2734.3	63.3	3.7	13.6	2.32	0.14	0.50
Cont. + 250 g/MT ABG	2941.9	69.2	4.1	14.5	2.35	0.14	0.50
Cont. + 750 g/MT ABG	2863.3	68.9	3.7	14.2	2.40	0.13	0.50

Table 3-4. Average BW, weight gain, phase mortality corrected FCR, and cumulative (Cum.) mortality corrected FCR of broilers receiving a 100x dose of Coccivac®-B and fed increasing levels of ABG (Experiment 2).

<table>
<tr><th rowspan="2">Treatment</th><th colspan="3">Body Weights</th><th colspan="2">Weight Gain</th><th colspan="3">Phase FCR</th><th>Cum. FCR</th></tr>
<tr><th>Day 10 (g)</th><th>Day 17 (g)</th><th>Day 20 (g)</th><th>Day 10-17</th><th>Day 17-20</th><th>Day 1-10</th><th>Day 10-17</th><th>Day 17-20</th><th>Day 1-20</th></tr>
<tr><td>Non-challenged Control</td><td rowspan="2">234.2^{bc}</td><td>530.2^{bc}</td><td>647.5^{bc}</td><td>310.7^{a}</td><td>445.4^{a}</td><td rowspan="2">1.184^{a}</td><td>1.283^{b}</td><td>1.691</td><td>1.299^{bc}</td></tr>
<tr><td>Control (Cont.)</td><td>544.4^{ab}</td><td>672.8^{ab}</td><td>304.2^{a}</td><td>426.2^{a}</td><td>1.422^{a}</td><td>1.675</td><td>1.355^{ab}</td></tr>
<tr><td>Cont. + 100 g/MT ABG</td><td>231.1^{c}</td><td>515.8^{c}</td><td>615.3^{c}</td><td>285.6^{b}</td><td>285.6^{b}</td><td>1.224^{a}</td><td>1.466^{a}</td><td>1.639</td><td>1.408^{a}</td></tr>
<tr><td>Cont. + 250 g/MT ABG</td><td>251.0^{a}</td><td>562.2^{a}</td><td>700.2^{a}</td><td>311.3^{a}</td><td>444.9^{a}</td><td>1.111^{b}</td><td>1.363^{ab}</td><td>1.561</td><td>1.284^{c}</td></tr>
<tr><td>Cont. + 750 g/MT ABG</td><td>244.4^{ab}</td><td>561.2^{a}</td><td>697.8^{a}</td><td>316.9^{a}</td><td>450.8^{a}</td><td>1.103^{b}</td><td>1.402^{a}</td><td>1.547</td><td>1.286^{c}</td></tr>
</table>

$^{a\text{-}c}$Means in columns differ at $p<0.05$

17-20. All other treatments experienced similar rates of body weight gain during the challenge period.

Inclusion of ABG at 250 and 750 g/MT in the control diet reduced ($p<0.05$) pre-challenge (d 1-10) mortality corrected FCR as compared to the control fed broilers (Table 3-4). While vaccine *Eimeria* challenge did not negatively impact body weight gain, mortality corrected FCR was increased ($p<0.05$) in control challenged broilers as compared to non-challenged broilers (d 10-17). During the same period, inclusion of 250 g/MT of ABG reduced FCR to a level comparable to the non-challenge control fed broilers while inclusion at 100 and 750 g/MT was similar to challenge control fed broilers. When evaluating cumulative FCR (d 1-20), inclusion of ABG at 250 and 750 g/MT significantly reduced ($p<0.05$) FCR as compared to the challenged control group to levels similar to the non-challenge control fed broilers.

Overall, lesion development associated with challenge was relatively low which may explain the lack of separation when evaluating body weight gain. Inclusion of ABG had no impact on upper intestinal lesion scores of *Eimeria* challenged broilers as compared to the challenged control group. All challenged broilers showed greater ($p<0.05$) average upper intestinal lesion scores as compared to the non-challenged control group (Table 3-5). Lesion development in the mid portion of the small intestine was extremely low, however, broilers fed 100 g/MT ABG were observed to have increased ($p<0.05$) mid intestinal lesion scores as compared to both control groups, although this represented only 2 broilers exhibiting lesions while no other treatments

Table 3-5. Intestinal lesion scores of male broilers 7 d post challenge receiving a 100x dose of Coccivac® B and fed increasing levels of ABG (Experiment 2).

Treatment	Upper	Middle	Lower
Non-challenged Control	0.00[b]	0.00[b]	0.04[b]
Control (Cont.)	1.10[a]	0.00[b]	0.34[ab]
Cont. + 100 g/MT ABG	1.27[a]	0.10[a]	0.30[ab]
Cont. + 250 g/MT ABG	1.07[a]	0.00[b]	0.50[a]
Cont. + 750 g/MT ABG	1.03[a]	0.00[b]	0.13[bc]

[a-c]Means in columns differ at $p<0.05$

expression any lesion development. Inclusion of ABG had no impact on average lower intestinal lesion scores as compared to the challenged control group.

Regarding oocyst output, the inclusion of ABG in the control diet had no impact on oocyst output 6, 7, 8, and 9 d post *Eimeria* challenge as compared to the challenged control group (Table 3-6). With regards to a four day post challenge average oocyst output, inclusion of ABG had no impact on average oocyst output as compared to the challenged control group. Inclusion of ABG at 250g/MT in the control group significantly lowered ($p<0.05$) four day average oocyst output as compared to the inclusion of ABG at 750 g/MT. All challenged broilers showed significantly greater ($p<0.05$) oocyst output as compared to the non-challenged control group 6, 7, 8, and 9 d post challenge and on a four day post challenge average oocyst output. There was a low level of oocyst shedding in one replicate pen of non-challenged control fed broilers indicating that horizontal transfer of the challenge organism did take place, however, broilers were reared in the same battery units as challenge organisms to allow for accurate evaluation and comparison of performance parameters to that of a non-challenge group.

Table 3-6. Oocyst output (oocysts/g of fecal material) of broilers receiving a 100x dose of Coccivac® B and fed increasing levels of ABG (Experiment 2).

Treatment	Days Post Challenge				Four day post challenge average
	Day 6	Day 7	Day 8	Day 9	
Non-challenged Control	563[c]	750[b]	188[b]	188[b]	422[c]
Control (Cont.)	31,500[ab]	16,050[a]	4,050[a]	4,200[a]	13,950[ab]
Cont. + 100 g/MT ABG	29,850[b]	13,950[a]	5,550[a]	5,550[a]	13,750[ab]
Cont. + 250 g/MT ABG	24,900[b]	15,450[a]	6,300[a]	4,050[a]	12,675[b]
Cont. + 750 g/MT ABG	44,100[a]	15,600[a]	3,600[a]	5,400[a]	17,175[a]

[a-c]Means in columns differ at $p<0.05$

Experiment 3. The inclusion of ABG had no impact on BW of Newcastle vaccinated broilers compared to the control group throughout the experiment (Table 3-7). With regards to FCR, the inclusion of ABG had no impact on FCR as compared to the control group throughout the experiment, however, the inclusion of ABG at 750 g/MT reduced ($p<0.05$) FCR from d 7-18 as compared to the inclusion of ABG at 250 g/MT (Table 3-7). Feed consumption (g/bird/day) was not effected with the inclusion of ABG as compared to the control group throughout the experiment (Table 3-8).

Newcastle specific antibody titers (Log_{10}) of Newcastle vaccinated broilers on d 25 were increased ($p<0.05$) in broilers fed ABG at 250 g/MT as compared to control fed broilers (Table 3-7).

Table 3-7. Average BW, phase mortality corrected FCR, cumulative mortality corrected FCR, and Newcastle Disease Virus antibody titers (Log_{10}) of broilers receiving a full dose of Newcastle vaccine and fed increasing levels of ABG (Experiment 3).

Treatment	Body Weights			Phase FCR			Cumulative FCR		Day 25 Newcastle Disease Antibody Titers
	Day 7 (g)	Day 18 (g)	Day 25 (g)	Day 1-7	Day 7-18	Day 18-25	Day 1-18	Day 1-25	
Control (Cont.)	144.5	559.8	926.8	1.177	1.455[ab]	1.692	1.395	1.517	1.833[b]
Cont. + 100 g/MT ABG	151.6	567.7	987.1	1.290	1.450[ab]	1.605	1.417	1.497	1.834[b]
Cont. + 250 g/MT ABG	145.4	550.4	956.2	1.201	1.472[a]	1.608	1.417	1.502	2.239[a]
Cont. + 750 g/MT ABG	142.3	575.9	968.2	1.228	1.382[b]	1.637	1.355	1.473	2.051[ab]

[a,b]Means in columns differ at $p<0.05$

Table 3-8. Feed Consumption (FC; grams of feed consumed per bird per day) of broilers receiving a full dose of Newcastle vaccine and fed increasing levels of ABG (Experiment 3).

Treatment	Phase FC			Cumulative FC	
	Day 1-7	Day 7-18	Day 18-25	Day 1-18	Day 1-25
Control (Cont.)	16.7	86.2	88.0	51.1	53.2
Cont. + 100 g/MT ABG	19.4	86.3	95.5	52.1	55.3
Cont. + 250 g/MT ABG	17.7	85.3	93.0	51.5	54.5
Cont. + 750 g/MT ABG	17.4	85.8	91.5	51.6	54.5

Discussion

In the present study, an algal β-(1,3)-glucan product (Algamune ZPC™) was supplemented to non-challenged and challenged broilers to assess the effect of an algae derived β-glucan in three consecutive experiments. On d 14, inclusion of ABG at 750 g/MT increased ($p<0.05$) early BW from 383.5 g in the control diet to 395.4 g. Similar benefits to early BW gains were reported when feeding a derivation of a β-(1,3)-(1,6)-glucan from *Agrobacterium sp.* R259 KCTC 10197B by Cho et al. (2013). The group reported BW gains from weeks 0 to 3 in broilers fed 0.1% β-glucan in a non-challenge setting. However, Chae et al. (2006) reported no differences in BW on d 17 when feeding a yeast derived β-glucan (YBG), indicating inconsistencies in reports on early BW effects when feeding varying sources of β-glucans. The inclusion of ABG in the current experiment had no impact on final BW at the conclusion of the experiment. Multiple publications have indicated no effect to final BW gains to broilers when fed YBG at varying levels in a non-challenge setting (Chae et al., 2006; Morales-Lopez et al., 2009; Cox et al., 2010a; and Zhang et al., 2012). Rathgeber et al. (2008) also reported no differences in BW when feeding YBG on d 14 and 24, but broilers receiving YBG on d 38 showed an increase in final BW as compared to the control groups. The use of ABG at 100 g/MT increased ($p<0.05$) FCR during the starter phase by nearly 6 points as compared to the control group. The impact on FCR can be explained by the smaller BW of broilers fed ABG at 100 g/MT at d 14, when feed consumption was not effected by treatment. Supplementation of ABG in the control diet had no effect on dietary phase FCR and cumulative FCR as compared to the control group for the remainder of the experiment, although decreasing ABG supplementation from 250 g/MT to 100 g/MT significantly reduced ($p<0.05$) FCR during the grower phase. The reduced impact of ABG on FCR is similar to reports by Chae et al., (2006), Rathgeber et al. (2008), Morales-Lopez et al. (2009), Cox et al., (2010a), and Zhang et al. (2012) who observed no impact on FCR when feeding YBG. Relative organ weights of the liver, spleen, and kidney were not impacted by feeding of ABG without the presence of a challenge. YBG was not found to impact relative liver weights by Morales-Lopez et al. (2009) and Zhang et al. (2012). Relative spleen weights have also been reported to be unaffected by YBG at levels as high as 0.1% by Rathgeber et al. (2008) and Cox et al. (2010). However, Zhang et al. (2012) reported a 3.9% increase in spleen weights in broilers fed YBG at similar levels.

It was the conclusion of Chae et al. (2006) that the effects dietary supplementation of β-glucan to broilers on performance is dependent on use in a challenge vs. non-challenge setting. For this reason two consecutive experiments were conducted evaluating ABG in the presence of an *Eimeria* disease challenge and following a Newcastle Virus vaccination program. Beta-glucan is thought to prime the immune system during infection, allowing the bird to sustain growth with no losses in performance (Cox et al., 2010b). For these reasons experiment two explored the use of ABG during an *Eimeria* challenge setting. At 10 days of age, all broilers supplemented with ABG were challenged with 100X dose of vaccine strain *Eimeria* oocysts (Coccivac®-B) containing *Eimeria acervulina*, *E. maxima*, *E. mivati*, and *E. tenella*. Half of the control group was also challenged, while the remaining half was kept as a non-challenged control group to allow for evaluation of performance associated with ABG. On d 10, prior to challenge, inclusion of ABG at 250 g/MT increased ($p<0.05$) BW by 17 g from the control group. These gains in early BW are similar to those observed in experiment 1. Significant gains ($p<0.05$) in BW continued post-challenge to d 20 in broilers supplemented ABG at 250 and 750 g/MT as compared to the non-challenged control group. Inclusion of ABG at 250 and 750 g/MT to the control diet also significantly reduced ($p<0.05$) d 10 FCR as compared to the control groups and reduced cumulative FCR for the entire experiment as compared to the challenged control group to levels similar to the non-challenge control group. These data are supported by the benefits of a whole yeast cell product provided to broilers by Shanmugasaundaram et al. (2013). On d 21 of the study, broilers were challenged with live coccidial oocysts. Broilers were supplemented with a whole yeast cell wall product as high as 0.2%, which increased BW gain and feed efficiency 12 d post coccidial challenge. Conversely to these findings, Cox et al. (2010b) observed no impact on performance when feeding YBG at levels up to 0.1% during an *Eimeria* challenge. The differences in the results between the two studies may be dose dependent since both are yeast based β-glucan. It could also be concluded that the differences in source of β-glucan impacted the differences in performance between these studies and the current experiment, and whether in a challenge or non-challenge setting.

The second experiment also evaluated the impact ABG during the *Eimeria* challenge on lesion scores and oocyst output. *Eimeria* disrupt intestinal cell linings such as the enterocyte layer, resulting in observable lesions. These lesions are associated with a reduction in nutrient absorption and reduced performance (Brake et al., 1997). Lesion scores were observed and recorded in broilers supplemented with ABG 7 d post *Eimeria* challenge. The inclusion of ABG in the control diet had no impact on average intestinal lesion score in the upper intestinal section as compared to the challenged control group although overall lesion development was extremely low, which may have prohibited the separation between ABG and control treatments. While challenge was not able to separate challenge and non-challenge control treatments based on body weight, the presence of *Eimeria* was sufficient to influence feed conversion ratio indicating significant damage associated with challenge. Feeding YBG at 0.1% has been reported to reduce upper and middle intestinal lesion scores by Cox et al. (2010b), although the same study found no differences in lower intestinal lesion scores in agreement with the current study. The current study also found no significant impact in oocyst output per gram of feces 6, 7, 8, or 9 d post challenge when broilers were supplemented with ABG, although numerical reductions were observed. However, supplementation of ABG at 250 g/MT significantly reduced the four d post challenge average of oocyst output as compared to supplementation of ABG at 750 g/MT, though no impact by ABG was observed as compared to the challenged control group. Lesions in the lower intestinal section and oocysts in the feces were detected in the non-challenged control group. Horizontal transfer of disease organism did take place in one replicate pen as treatments were randomly distributed in block design in stacked battery cages to allow for comparison of performance parameters to non-challenge control broilers. The effects of ABG on oocyst output is supported by Shanmugasundaram et al. (2013), who reported no effect on fecal oocyst output 5 and 12 d post *Eimeria* challenge when feeding whole yeast cell product at 0.1 and 0.2%. A reduction in fecal oocyst counts was reported by Shanmugasundaram et al. (2013) 7 d post challenge, indicating β-glucan can reduce oocyst output, but that source and dosage may play a role in the timing of oocyst reduction.

The third and final experiment evaluated the effects of ABG during a Newcastle vaccination program. Broilers were fed ABG in a starter diet to 18 d post vaccination. A vaccine boost was administered on d 18, and ABG was removed from the diet. Inclusion of ABG had no impact on performance parameters as was observed at the conclusion of Experiments 1 and 2, although this may be associated with the lower number of replicates and total birds placed. An et al. (2008) evaluated the effects of YBG in a 35 d growout after administering a Newcastle virus and infectious bronchitis virus vaccine. The group reported an increase in BW and improvement in FCR when YBG was supplemented as high as 0.1%. Inclusion of ABG at 250 g/MT increased ($p<0.05$) d 25 antibody titer levels as compared to the control diet. The increase in antibody titers after removal of ABG from the diet suggests that β-glucan primes the immune system when fed in a diet. This was also observed by Cox et al., (2010b) when a yeast β-glucan was fed during an *Eimeria* challenge. These observations are supported by An et al. (2008) who also observed an increase in d 35 Newcastle virus antibody titers when feeding YBG at 0.05 and 0.1%, although not at 0.025%, suggesting dosage level of β-glucan impacted the increase in antibody titers.

These data confirm that an algal derived β-(1,3)-glucan can improve early performance parameters in a non-challenge setting, increase Newcastle virus specific antibody titers, and eliminate growth performance reductions in *Eimeria* challenged broilers . These data also confirm that dosage level of β-glucan plays a significant role in performance effects in both non-challenged and challenged settings. Results of these experiments confirm the statements by Chae et al. (2006) that variable effects of β-glucan on performance occur in a challenge vs. non-challenge setting. Inclusion rates and dosage of ABG to maximize observational effects seems to differ from YBG, which can be attributed to the difference in β-glucan structures associated with source. These three experiments demonstrate the effectiveness of ABG in poultry production although additional research must be conducted to further confirm proper dosage level as the majority of research in this area has focused on yeast derived β-glucan.

CHAPTER IV

EVALUATION OF THE INCLUSION OF A NOVEL THERMOSTABLE XYLANASE IN BROILER DIETS CONTAINING WHEAT AND DDGS

Introduction

The use of wheat and dried distillers' grains with solubles (DDGS), a by-product of ethanol production, as primary ingredients in U.S. poultry diets has been hampered by their high non-starch polysaccharide (NSP) content. NSP's are a major component of dietary fiber comprised of cellulose and non-celluclitic polysaccharides. Of the numerous NSP's known, arabinose and xylose (arabinoxylans), β-(1,3)-(1,4)-glucans, and cellulose are NSPs of concern in diets manufactured with wheat and DDGS (Henry, 1987; Slominski et al., 2000; Widyaratne and Zijlstra, 2007). The presence of NSP, which are indigestible by monogastric species, results in reductions in nutrient utilization (Meng et al., 2005).

The anti-nutritive properties of NSPs include the inhibition of ileal digestibility of nutrients, reduced diet AME values, increases in intestinal viscosity, reduction in nutrient digestibility and utilization, and overall reductions in broiler performance (Pettersson and Aman, 1988; Annison, 1993; Choct, 2006). An increase in viscosity in the small intestine causes a decrease in contact between digestive enzymes, leading to a decrease in nutrient absorption and broiler performance (Choct et al., 1995). The presence of NSP in the cell wall of grains has also been noted to entrap starch, protein, and other nutrients within grain, yielding them indigestible by broilers (Theander et al., 1989; Bedford and Autio, 1996; Wiseman et al., 2000).

Pelleting of poultry feeds during manufacturing is accepted for its reductions in feed wastage and moderate improvements in feed efficiency. Feed is subjected to many heat sources during manufacture, especially during grinding of grains, conditioning, and pelleting/crumbling. Feed is conditioned and pelleted at high heat using steam in the conditioner to increase pellet quality (Abdollahi et al., 2010). Pelleting wheat diets for poultry at elevated temperatures over 60°C has been reported to impact broiler performance as compared to corn diets through increases in diet and digesta viscosity (Abdollahi et al., 2010). Increases in previously encapsulated NSP has been observed when pelleting wheat based diets at elevated temperatures, resulting in overall decreases in performance parameters (Coweison et al., 2005; Abdollahi et al., 2010). Abdollahi et al. (2010) also reported a decrease in ileal digestibility of starch and protein in broilers when manufacturing wheat based feeds at elevated temperatures.

The use of supplemental carbohydrases in broiler diets to improve performance and decrease the effect of NSPs is widely accepted. Carbohydrases degrade high molecular weight polysaccharides such as arabinoxylan to simple sugars, oligosaccharides, and lower molecular weight polysaccharides (Slominski et al., 1993; Castanon et el., 1997). The inclusion of exogenous xylanase in broiler diets containing wheat has been extensively reviewed for more than two decades. Xylanase cleaves and hydrolyzes the xylose backbone of arabinoxylans, allowing access to entrapped nutrients and increasing overall utilization of the diet by the bird (Choct and Annison, 1992;

Coweison et al., 2005; Meng et al., 2005). The increases in digesta viscosity associated with wheat and viscous grain diets and diets manufactured at elevated temperatures can be off-set with the use of supplemental xylanase. Xylanase reduces intestinal viscosity through degradation of soluble arabinoxylans, reducing gut viscosity, leading to improvements in nutrient utilization and performance uniformity (Choct and Annison, 1992; Choct et al., 1999; Coweison et al., 2005). These improvements are associated with observations in improvements of performance by broilers in terms of daily gain, feed efficiency, and increases in ileal digestibility of nutrients (Choct et al., 2004; Wang et al., 2005). Advances in enzyme technology including thermostability allow for products to be added to the mash feed in the mixer prior to pelleting. Pelleting wheat diets for broilers supplemented with xylanase at elevated temperatures up to 80°C has been reported to maximize broiler performance compared to more extreme temperatures (Silverside and Bedford, 1999), although Cowieson et al. (2005) reported benefits to performance in diets manufactured at 90°C. The objective of the current study was to determine the effect of increasing xylanase levels in diets containing wheat and DDGS on observed growth performance and ileal digestible energy at elevated pelleting temperatures in two separate experiments.

Materials and Methods

Experimental Design. Two consecutive experiments were conducted to evaluate the effect of a novel xylanase when pelleted at elevated temperatures during a 38 d growout period and a 21 d battery study. The xylanase enzyme was originally isolated from an environmental soil sample. The wild-type enzyme is a thermophilic glycoside hydrolase family (GH) 11 xylanase with a melting temperature (T_m) of 76°C (measured by differential scanning calorimetry). This wild-type xylanase was further optimized using directed evolution technology to create a hyper-thermostable variant with a $T_m >$ 100°C. Xylanase was included at levels of 0, 250, 500, 1,000, and 2,000 U/kg for both experiments. One unit (U) of activity is defined as 1 µmole of hydrolyzed xylan substrate per minute at 50°C and pH 5.3. Experiments were conducted under the Texas A&M Guidelines for animal care. Broilers were provided age appropriate heat and ventilation and given access to feed and water *ad libitum*.

Experiment 1. A 38 d growout experiment was conducted to evaluate the effects of dietary inclusion of xylanase on broiler growth performance and digestibility of energy when diets are pelleted at 82°C. On d of hatch, 1,750 male broilers were weighed, wing banded, and allotted to floor pens and dietary treatments based on body weight. Thirty-five broilers were placed per replicate pen, with 10 replicate pens per treatment, for a total of 50 replicate pens. Stocking density was set to 0.9 sq. ft./bird. Broilers and feed were weighed on d 14, 28, and 38 (at the end of each dietary phase) for the calculation of body weights (BW) and mortality corrected feed conversion ratio (FCR). On d 38, three broilers per replicate pen were randomly selected and euthanized, with ileal contents removed and pooled per replicate pen for the determination of ileal digestible energy (IDE).

Experiment 2. A 21 d battery experiment was conducted to evaluate the effects of dietary inclusion of xylanase on early broiler growth performance, ileal digestible energy, and enzymatic recovery when diets were pelleted at 92°C. On d of hatch, 360 male broilers were weighed, wing banded, and randomly allotted to battery pens and dietary treatments based on body weight. Six broilers were placed per replicate pen, with 12 replicate pens per treatment, for a total of 60 replicate pens. Broilers and feed were weighed on d 7, 14, and 21 for the calculation of BW and FCR. On d 21, all broilers per replicate pen were euthanized. Ileal contents were collected and pooled per replicate pen for the determination of IDE.

Experimental Diets. Broilers for both experiments were fed a diets containing wheat and DDGS meeting the nutrient specifications outlined in Table 4-1 and 4-2. Dietary formulations included wheat and DDGS at 30 and 15%, respectively. Titanium dioxide was included at 0.4% of the diet, at the expense of corn, and utilized as an indigestible marker for the determination of IDE. One large basal diet was manufactured for each dietary phase and experiment to eliminate nutrient variability between treatments and divided into five equal parts. Xylanase was included over the top to the basal diet during mixing and prior to pelleting at levels of 0, 250, 500, 1,000, and 2,000 U/kg. In Experiment 1, broilers were fed a starter (d1-14), grower (14-28), and finisher (28-38) diet pelleted at 82°C. An identical starter diet to Experiment 1 was manufactured for Experiment 2. The diet was pelleted at 92°C and was fed from d 1-21. Mash feed was steam conditioned for 30 seconds, with temperatures monitored within the conditioner and as the feed exited the pelleter. Grower and finisher feeds were fed as a pellet, where starter feeds were crumbled post pelleting. Mash and pelleted samples of all diets and treatments were collected and analyzed for nutrient content (Table 4-1 and 4-2) and xylanase recovery (Table 4-3 and 4-4). Crude protein was determined by

Table 4-1. Dietary formulation and calculated and analyzed nutrient content of diets, based on percentages, for male broilers fed xylanase at increasing levels and pelleted at 82°C (Experiment 1).

Ingredient	Starter	Grower	Finisher
Whole Wheat	30.00	30.00	30.00
Low Oil-Dried Distillers Grain with Solubles[1]	15.00	15.00	15.00
Corn	18.79	21.62	32.78
Dehulled Soybean Meal (48%)	27.67	25.41	14.38
DL-Methionine (99%)	0.19	0.19	0.22
Lysine HCL	0.24	0.16	0.42
L-Threonine	-	-	0.08
Fat, A/V Blend	4.33	4.20	3.20
Limestone	1.63	1.55	1.47
Monocalcium Phosphate	1.41	1.14	1.07
Sodium Chloride	0.39	0.39	0.13
Sodium Bicarbonate	-	-	0.35
Vitamin Premix[2]	0.25	0.25	0.25
Trace Minerals[3]	0.05	0.05	0.05
Titanium Dioxide	-	-	0.40
Coban 90[4]	0.05	0.05	0.05
Calculated Nutrient Content			
Protein	23.33	22.39	18.34
Lysine	1.27	1.15	1.05
Methionine	0.55	0.54	0.51
TSAA	0.93	0.90	0.82
Threonine	0.81	0.78	0.69
Arginine	1.35	1.30	1.10
Valine	1.04	1.00	0.80
Calcium	0.95	0.87	0.80
Available Phosphorus	0.47	0.41	0.38
Sodium	0.20	0.20	0.20
Metabolizable Energy (kcal/kg)	3000	3025	3075
Analyzed Nutrient Content			
Moisture	9.82	9.60	12.00
Crude Protein	24.3	24.5	17.7
Crude Fat	6.56	5.05	5.95
Acid Detergent Fiber	3.34	3.69	3.50
Ash	5.03	5.59	5.81

[1] Analyzed Nutrient Content of LO-DDGS: Moisture 10.92%, Crude Protein 29.02%, Metabolizable Energy 2354 kcal/kg, Crude Fat 4.86%, Acid Detergent Fiber 7.70%, and Ash of 5.18%

[2]Vitamin premix added at this rate yields 11,023 IU vitamin A, 3,858 IU vitamin D_3, 46 IU vitamin E, 0.0165 mg B_{12}, 5.845 mg riboflavin, 45.93 mg niacin, 20.21 mg d-pantothenic acid, 477.67 mg choline, 1.47 mg menadione, 1.75 mg folic acid, 7.17 mg pyroxidine, 2.94 mg thiamine, 0.55 mg biotin per kg diet. The carrier is ground rice hulls.

[3] Trace mineral premix added at this rate yields 149.6 mg manganese, 125.1 mg zinc, 16.5 mg iron, 1.7 mg copper, 1.05 mg iodine, 0.25 mg selenium, a minimum of 6.27 mg calcium, and a maximum of 8.69 mg calcium per kg of diet. The carrier is calcium carbonate and the premix contains less than 1% mineral oil.

[4] Active drug ingredient monensin sodium, 90 g/lb (90 g/ton inclusion; Elanco Animal Health, Indianapolis, IN). As an aid in the prevention of coccidiosis caused by *Eimeria necarix*, *Eimeria tenella*, *Eimeria acervulina*, *Eimeria brunette*, *Eimeria mivati*, and *Eimeria maxima*

Table 4-2. Dietary formulation and calculated and analyzed nutrient content of the starter diet, based on percentages, for male broilers fed xylanase at increasing levels and pelleted at 92°C (Experiment 2).

Ingredient	Starter
Whole Wheat	30.00
Low Oil-Dried Distillers Grain with Solubles[1]	15.00
Corn	18.39
Dehulled Soybean Meal (48%)	27.67
DL-Methionine (99%)	0.19
Lysine HCL	0.24
Fat, A/V Blend	4.33
Limestone	1.63
Monocalcium Phosphate	1.41
Sodium Chloride	0.39
Sodium Bicarbonate	-
Vitamin Premix[2]	0.25
Trace Minerals[3]	0.05
Titanium Dioxide	0.40
Coban 90[4]	0.05
Calculated Nutrient Content	
Protein	23.33
Lysine	1.27
Methionine	0.55
TSAA	0.93
Threonine	0.81
Arginine	1.35
Valine	1.04
Calcium	0.95
Available Phosphorus	0.47
Sodium	0.20
Metabolizable Energy (kcal/kg)	3000
Analyzed Nutrient Content	
Moisture	9.82
Crude Protein	24.3
Crude Fat	6.56
Acid Detergent Fiber	3.34
Ash	5.03

[1]Analyzed Nutrient Content of LO-DDGS: Moisture 10.92%, Crude Protein 29.02%, Metabolizable Energy 2354 kcal/kg, Crude Fat 4.86%, Acid Detergent Fiber 7.70%, and Ash of 5.18%.
[2]Vitamin premix added at this rate yields 11,023 IU vitamin A, 3,858 IU vitamin D_3, 46 IU vitamin E, 0.0165 mg B_{12}, 5.845 mg riboflavin, 45.93 mg niacin, 20.21 mg d-pantothenic acid, 477.67 mg choline, 1.47 mg menadione, 1.75 mg folic acid, 7.17 mg pyroxidine, 2.94 mg thiamine, 0.55 mg biotin per kg diet. The carrier is ground rice hulls.
[3] Trace mineral premix added at this rate yields 149.6 mg manganese, 125.1 mg zinc, 16.5 mg iron, 1.7 mg copper, 1.05 mg iodine, 0.25 mg selenium, a minimum of 6.27 mg calcium, and a maximum of 8.69 mg calcium per kg of diet. The carrier is calcium carbonate and the premix contains less than 1% mineral oil.
[4] Active drug ingredient monensin sodium, 90 g/lb (90 g/ton inclusion; Elanco Animal Health, Indianapolis, IN). As an aid in the prevention of coccidiosis caused by *Eimeria necarix*, *Eimeria tenella*, *Eimeria acervulina*, *Eimeria brunette*, *Eimeria mivati*, and *Eimeria maxima*.

Table 4-3. Xylanase recovery for wheat-DDGS diets pelleted at 82°C (Experiment 1).

Target Dose (U/kg)	Dietary Phase	Xylanase Recovery (U/kg)
0	Starter	0
	Grower	0
	Finisher	0
250	Starter	176
	Grower	275
	Finisher	372
500	Starter	514
	Grower	388
	Finisher	519
1,000	Starter	1,059
	Grower	1,010
	Finisher	906
2,000	Starter	1,733
	Grower	1,505
	Finisher	1,703

Table 4-4. Xylanase recovery for a wheat-DDGS starter diet pelleted at 92°C (Experiment 2).

Target Dose (U/kg)		DNS
0	Mash	0
	Pellet	0
250	Mash	149
	Pellet	104
500	Mash	495
	Pellet	452
1,000	Mash	326
	Pellet	421
2,000	Mash	2,821
	Pellet	1,701

combustion using an AOAC (AOAC, 2000) method (AOAC method 990.03), total phosphorus was determined by wet ash inductively coupled plasma spectroscopy (AOAC method 985.01M), ADF was determined using an Ankom digestion unit (AOAC method 973.18) (Ankom Technology, Macedon, NY), and an ether extraction method was used to determine crude fat (AOAC method 920.39). The activity of the xylanase was determined by analyzing the amount of reducing sugars during enzymatic hydrolysis of xylan from beechwood. Activity was determined spectrophotometrically following color development with 3,5-Dinitrosalicylic Acid (DNS) buffer and compared to a xylose standard curve.

Ileal Digestible Energy (IDE). IDE was determined at the conclusion of both experiments using titanium dioxide as an indigestible marker. Ileal contents were removed four centimeters posterior to Meckle's Divereticulum and four centimeters anterior to the ileal-cecum junction and pooled per replicate pen. Ileal and feed samples were dried at 100°C for 24 hours. Samples were ground for gross energy and titanium concentration determination.

Titanium concentration was determined using a modified protocol outlined by Short et al. (1996). Half a gram of each dried sample was weighed and placed in an ashing oven at 450°C. Following ashing, each sample was titrated with 10 mL of 7.4 M sulfuric acid and boiled at 200°C for 3 hours until dissolved. Samples were then titrated with 10 mL of 30% hydrogen peroxide. Total sample volume of 100 mL was achieved using distilled water. Samples were analyzed for absorption using a Thermo Fisher Scientific Genesys 10S UV-Vis (Model 10S UV-Vis) Spectrophotometer at 410 nm.

Gross energy of feed and ileal samples wsd determined using a Parr 6400 bomb calorimeter. IDE was calculated using the following equation (Scott et al., 1982):

$$\text{Gross } E_f - \text{Excreta } E_i \text{ where } E_i = GE \times (Ti_f/Ti_i)$$

Statistical Analysis

Body weights, mortality, feed consumption, FCR, IDE, and IDEc were analyzed *via* Analysis of Variance (ANOVA) using the General Linear Model Procedure using SPSS V 18.0. Mortality data was subjected to an arc sin transformation prior to analysis. Means were deemed significantly different at $p \leq 0.05$ and separated using Duncan's Multiple Range Test. The experimental unit for each trait evaluated was pen.

Results

Experiment 1. On d 14, the inclusion of xylanase in the wheat-DDGS diet pelleted at 82°C had no impact on body weight (kg; BW) as compared to the control diet (Table 4-5). The inclusion of xylanase at 1,000 and 2,000 U/kg increased ($p<0.05$) BW as compared to the control diet on d 28, with the 250 and 500 U/kg inclusion rates being intermediate. At the conclusion of the study, the inclusion of xylanase had no impact on BW as compared to the control. Additionally, no impact was observed on mortality throughout the experiment.

Consistent improvements were observed in FCR with the inclusion of xylanase. During the starter phase (d 1-14), inclusion of xylanase at 500, 1,000, and 2,000 U/kg reduced ($p<0.05$) FCR as compared to the control diet, with the 250 U/kg treatment being intermediate (Table 4-5). During the grower and finisher phases, the inclusion of xylanase at all evaluated levels reduced ($p<0.05$) FCR as compared to the control diet.

Table 4-5. Body weights, dietary phase mortality corrected FCR, cumulative mortality corrected FCR, mortality (Mort.), and IDE of male broilers fed increasing levels of xylanase in wheat-DDGS diets pelleted at 82°C (Experiment 1).

Treatment (U/kg xylanase)	Body Weights			Dietary Phase FCR			Cumulative FCR		Mort (%)	IDE
	Day 14 (g)	Day 28 (kg)	Day 38 (kg)	Starter	Grower	Finisher	Day 1-28	Day 1-38		
0	505.3	1.598[b]	2.500	1.292[a]	1.600[a]	2.204[a]	1.508[a]	1.760[a]	1.71	3,004[b]
250	514.8	1.629[ab]	2.536	1.261[ab]	1.548[b]	2.116[b]	1.462[b]	1.698[b]	3.14	3,274[a]
500	507.0	1.613[ab]	2.523	1.249[b]	1.553[b]	2.117[b]	1.462[b]	1.699[b]	2.86	3,340[a]
1000	511.9	1.642[a]	2.558	1.256[b]	1.555[b]	2.112[b]	1.466[b]	1.698[b]	3.14	3,143[ab]
2000	516.4	1.639[a]	2.541	1.251[b]	1.532[b]	2.095[b]	1.448[b]	1.680[b]	1.71	3,271[a]

[a,b]Means in columns differ at $p<0.05$

Similar to the grower and finisher phases, xylanase inclusion at all evaluated levels reduced ($p<0.05$) FCR as compared to the control diet on d 1-28 and d 1-38.

The improvements in FCR can be attributed to increases in ileal digestible energy (IDE) with the inclusion of xylanase. Inclusion of xylanase at 250, 500, and 2,000 U/kg in the control diet increased ($p<0.05$) IDE as compared to the control diet (Table 4-5).

Experiment 2. The inclusion of xylanase did not impact BW throughout the experiment as compared to the control diet (Table 4-6). Similar to Experiment 1, xylanase inclusion did not impact on mortality as compared to the control diet (Table 4-6).

Feed consumption (grams of feed per bird per day; FC) was influenced with xylanase inclusion resulting in FCR differences during the experiment. Xylanase inclusion at 1,000 U/kg reduced ($p<0.05$) FC from d 1-7 as compared to the control diet (Table 4-7). On d 14-21, the inclusion of xylanase at 500, 1,000, and 2,000 U/kg reduced ($p<0.05$) FC as compared to the control diet. At the conclusion of the experiment, FC from d 1 -21 was reduced ($p<0.05$) with the inclusion of xylanase at 500 and 2,000 U/kg to the wheat-DDGS diet pelleted at 92°C as compared to the control diet.

Regarding weekly mortality corrected FCR, the inclusion of xylanase at all levels in diets containing wheat and DDGS pelleted at 92°C significantly reduced ($p<0.05$) FCR compared to the control diet on d 1- and d 14-21 (Table 4-7). With regards to cumulative mortality corrected FCR for d 1-14, inclusion of xylanase at 500, 1,000, and 2,000 reduced ($p<0.05$) FCR as compared to the control diet. At the conclusion of the experiment, the inclusion of xylanase at all levels to the wheat-DDGS diet pelleted at 92°C reduced ($p<0.05$) total cumulative FCR as compared to the control diet. As in Experiment 1, improvements in FCR can be attributed to increases in IDE. Xylanase inclusion increased ($p<0.05$) IDE in broilers fed diets supplemented with 500, 1,000, and 2,000 U/kg of xylanase (Table 4-6).

Table 4-6. Body weights, weekly mortality corrected FCR, cumulative mortality corrected FCR, mortality, and IDE of male broilers fed increasing levels of xylanase in wheat-DDGS diets pelleted at 92°C (Experiment 2).

Treatment (U/kg xylanase)	Body Weights			Weekly FCR			Cumulative FCR		Mort (%)	IDE
	Day 7 (g)	Day 14 (g)	Day 21 (g)	Day 1-7	Day 7-14	Day 14-21	Day 1-14	Day 1-21		
0	151.9	440.4	897.6	1.388[a]	1.398[ab]	1.523[a]	1.394[a]	1.460[a]	4.20	3,346[b]
250	159.5	457.1	925.9	1.288[b]	1.407[a]	1.443[b]	1.372[ab]	1.409[b]	4.20	3,451[ab]
500	158.3	438.5	909.8	1.281[b]	1.343[b]	1.430[b]	1.323[c]	1.308[b]	2.80	3,547[a]
1000	151.1	434.9	897.1	1.254[b]	1.347[b]	1.456[b]	1.320[c]	1.393[b]	1.40	3,530[a]
2000	158.6	445.5	912.4	1.275[b]	1.376[ab]	1.444[b]	1.346[bc]	1.396[b]	6.90	3,505[a]

[a-c]Means in columns differ at $p<0.05$

Table 4-7. Feed Consumption (FC; grams of feed consumed per bird per day) of male broilers fed increasing levels of xylanase in wheat-DDGS diets pelleted at 92°C (Experiment 2).

Treatment	Weekly FC			Cumulative FC	
	Day 1-7	Day 7-14	Day 14-21	Day 1-14	Day 1-21
Control (Cont.)	21.6[a]	57.5[ab]	99.5[a]	39.5[ab]	59.2[a]
Cont. + 250 U/kg xylanase	21.4[a]	58.9[a]	96.5[ab]	40.0[a]	58.5[ab]
Cont. + 500 U/kg xylanase	20.8[ab]	53.0[b]	96.1[b]	36.7[b]	56.2[b]
Cont. + 1,000 U/kg xylanase	19.5[b]	54.6[ab]	96.1[b]	37.0[b]	56.7[ab]
Cont. + 2,000 U/kg xylanase	20.3[ab]	55.9[ab]	94.7[b]	37.7[ab]	56.3[b]

[a,b]Means in columns differ at $p<0.05$

Discussion

It is well established that viscous grains such as wheat and DDGS contain high concentrations of NSP, mostly arabinoxylan. The NSP content of these grains cause reductions in broiler performance and nutrient utilization when these grains are used in poultry diets at elevated levels. These reductions have been linked to increases in digesta viscosity (Choct and Annison, 1992; Cowieson et al., 2005). High thermal feed manufacture of viscous grains also leads to increases in viscosity of finished feed (Cowieson et al., 2005; Abdollahi et al., 2010). An increase in digesta viscosity causes a reduction in rate of feed passage, decrease in contact between digestive enzymes and their substrate, and decreases in nutrient digestion, absorption, and utilization (Annison, 1993; Choct et al., 1995; Coweison et al., 2005). Supplemental carbohydrases have been used to degrade high molecular weight polysaccharides (Slominksi et al., 1993; Castanon et al., 1997). Specifically, xylanase hydrolyzes insoluble arabinoxylan and increases nutrient availability (Choct and Annison, 1992; Cowieson et al., 2005). Improvements in BW and FCR are thus associated with enzyme supplementation and reductions in viscosity (Meng et al., 2005; Cowieson and Massey O'Neill, 2013).

During Experiment 1, final BW was not impacted by the supplementation of xylanase when the diet was pelleted at 82°C. A 2.7% increase in BW occurred with xylanase inclusion from 1,000 and 2,000 U/kg, although no BW differences between the control and enzyme supplemented diet was observed at the conclusion of the experiment. The second experiment also resulted in no significant impact on BW with the supplementation of xylanase when the diet was pelleted at 92°C. The lack of positive influence on BW with xylanase supplementation in diets containing wheat was also reported by Cowieson et al. (2005). The report observed no impact on broiler BW by xylanase supplementation at 2,000 U/kg when a diet containing 53-65% wheat was manufactured at 80°C. However, the report goes on to state that pelleting the same diet with xylanase at 85 and 90°C significantly improved BW gain compared to the non-supplemented diets. This is contradictory to the previous findings of Silversides and Bedford (1999) whose regression analysis stated that BW gain of xylanase-supplemented wheat diets reaches a maximum when the diet is pelleted between 80 and 85°C.

During Experiment 2, the use of supplemental xylanase at 500 and 1,000 U/kg in the diet manufactured at 92°C reduced cumulative feed consumption over 7 and 5% on d 14 and 21, respectively. The reduction in feed intake resulted in an overall decrease in FCR in the battery experiment, and offers an explanation for the reduction in FCR during the 38 d growout experiment. The improvement in FCR with the inclusion of xylanase in wheat and other viscous grains has been observed in numerous studies (Choct et al., 2004; Wang et al., 2005; Nian et al., 2011b; Cowieson and Masey O'Neill, 2013). During Experiment 1, the inclusion of xylanase at 500 U/kg and higher improved FCR by 4, 5, and 9 points in the starter, grower, and finisher phases, respectively, as compared to the control group. Cumulatively, the use of xylanase at levels of 250 U/kg and above reduced FCR in the diet manufactured at 82°C. Xylanase inclusion also benefited FCR when the diet was pelleted at 92°C. During Experiment 2, inclusion of xylanase at levels of 500 U/kg and above again improved FCR on weekly intervals starting at d 7. No influence on FCR was observed by xylanase inclusion at 250 U/kg until the weekly interval from d 14-21. At the end of the experiment, cumulative d 21 FCR was reduced by xylanase inclusion at all levels.

The current study falls in line with the published literature in terms of improvements to FCR when using xylanase in diets containing wheat and pelleted at increased temperatures (Silversides and Bedford, 1999; Cowieson et al., 2005). Though not evaluated in the current study, the reduction in digesta viscosity by supplementation of xylanase has been attributed to benefits in performance of broilers fed diets containing wheat and manufactured at high temperatures (Cowieson et al., 2005). The combination of the high concentration of arabinoxylan and increased pelleting temperature has been connected to an increase in intestinal viscosity in broilers fed diets containing wheat (Choct and Annison, 1992; Cowieson et al., 2005). Increased pelleting temperature can also increase the solubility of NSP and destroy endogenous enzymes, overall increasing digestive viscosity (Nissinen, 1994; Silversides and Bedford, 1999). The increase in digesta viscosity decreases the contact between digestive enzymes and their substrates, reduces rate of feed passage, increases water consumption, and increases proliferation of bacteria in the gastrointestinal track, leading to overall decreases in nutrient digestion, absorption, and utilization (Annison, 1993; Choct et al, 1995; Cowieson et al., 2005). Supplementation of enzymes by Silversides and Bedford (1999) maximized viscosity reduction when increasing pelleting temperature to 95°C, which resulted in best performance in the experiment. The effectiveness of xylanase at different pelleting temperatures could explain the differences in dosage effect between the two experiments in the current study. When pelleted at 82°C, xylanase dosage at 250 U/kg had no significant impact on FCR in the starter phase of the experiment. It was not until the grower phase and cumulatively on d 28 that xylanase inclusion at the lowest level impacted FCR. However, in the second experiment, when the diet was manufactured at 92°C, the effectiveness of xylanase at a dosage of 250 U/kg was immediate from d 1-7 and d 14-21 in reducing FCR as compared to the control diet, though not cumulatively from d 1-14. This suggests that at higher manufacture temperatures the required dosage of xylanase may be lower. The findings by Bedford et al. (1997) state that an increase in enzyme effectiveness at higher processing temperatures could be due to increased substrate being made available for enzyme degradation or to enzymatic degradation of NSP occurring during diet manufacture. Thus at a higher manufacture temperature as in experiment two a lower dosage and thus lower enzyme activity was required to effectively reduce FCR due to the increase in substrate available for hydrolysis and the degradation of NSP.

The NSP in the endosperm cell wall of viscous grains act as a physical barrier in feed ingredients, entrapping starch and protein and preventing utilization by broilers (Theander et al., 1989; Bedford and Autio, 1996; Wiseman et al., 2000). Exogenous xylanase cleaves the xylose backbone of arabinoxylans of viscous grains, breaking down the cell wall, allowing for a release of entrapped nutrients (Bedford, 2000; Meng et al., 2005). Enzyme inclusion in diets increases the rate of nutrient digestibility as well as moves the site of digestion and absorption of starch and protein in the anterior end of the digestive track (Bedford, 2000). Because starch is the major contributor to the energy value of the diet, the current study evaluated IDE to quantify the energy releasing capacity with xylanase inclusion. The inclusion of xylanase significantly increased IDE by a range of 267-336 kcal/kg in diets manufactured at 82°C on d 38. The inclusion of xylanase increased d 21 IDE by a range of 105-201 kcal/kg in diets manufactured at 92°C, though not significantly until a dosage of 500 U/kg or higher. These data are supported by the findings of Cowieson and Masey O'Neill (2013) who reported significant increases in d 28 IDE of 83 kcal/kg and d 49 of 214 kcal/kg. Choct et al. (1999) also reported an increase in starch digestibility in the ileum with the supplementation of enzymes. These reports show the effectiveness of xylanase to target its specific substrate within the digesta, allowing for breakdown of the xylose backbone of grain cell walls and releasing entrapped starch. The released starch improves utilization, and in the case of the current experiments, allowed for a decrease in feed intake while maintaining similar BW to the control diets. It is also important to note that IDE was affected greater by xylanase inclusion in older birds, as noted in the greater range in IDE increases in Experiment 1 as compared to Experiment 2, in agreement with the conclusions of Cowieson and Masey O'Neill (2013).

These data confirm the inclusion of supplemental xylanase in broiler diets containing wheat and DDGS had no effect on final BW when the diets were pelleted at 82 and 92°C. However, inclusion of xylanase at 500 U/kg consistently reduced feed intake and FCR regardless of pelleting temperature. These benefits to performance can be attributed to increased release of starch by xylanase as seen in the increases in IDE of broilers at 21 and 38 d of age.

CHAPTER V

EVALUATION OF THE INCLUSION OF A NOVEL THERMOSTABLE XYLANASE IN BROILER CORN-SOY DIETS

Introduction

Feed cost is the largest expense to broiler integrators during production of poultry meat, and is directly tied to the fluctuation in cost of finished market products incurred by consumers. With the goal of the industry to be a cheap source of protein for consumers, nutritionists must maximize nutrient utilization and improve feed efficiency with ingredients on hand (Williams et al., 2014).

Globally, corn is the most abundantly grown cereal crop available to animal agriculture (Cowieson, 2005), with the National Corn Growers Association (2013) reporting global corn production of 33,582 million bushels of corn produced for the 2012-2013 production cycle. Although corn on average has a relatively high starch content of 65% (NCGA, 2013), corn obtained from different locations, seasons, and years indicate various levels of nutrient composition (Leeson and Summers, 1976; Maier, 1995; Collins et al., 1998). Thus, the nutritional value of corn as a feed ingredient for poultry is a function of the starch, oil, protein, and antinutrient content of the corn source (Cowieson, 2005). It is important for nutritionists to account for this variability in corn matrix profiles to maximize nutrient digestibility and utilization in broilers. Ileal digestibility of corn starch has been reported to peak at 85% (Noy and Sklan, 1994), suggesting that undigested starch may limit the energy value of corn.

It has been suggested that undigested nutrients such as starch may be hindered by antinutrient factors in corn. Non-starch polysaccharides (NSPs) have been noted to act as a physical barrier to endogenous enzymes, encapsulating starch and protein and making them unavailable to broilers (Gracia et al., 2003; Cowieson, 2005; Choct, 2006; Slominski, 2011). Compared to wheat, corn has a relatively low NSP content, with a total and water-soluble NSP content of 76.3 and 6.4 mg/g, respectively (Meng and Slominski, 2005). Arabinoxylan and β-glucan account for the majority of corn cell wall carbohydrates (Classen, 1996), with arabinoxylan accounting for 5.8% (Nian et al., 2011a). Although low in NSP, an estimated 400-450 kcal of energy per kg of diet goes undigested by broilers in corn-SBM diets (Cowieson, 2010). The loss of energy to undigested fat, starch, and protein presents an opportunity to use exogenous enzymes to make this energy available to birds (Cowieson, 2010).

The use of exogenous carbohydrases in diets as degrading enzymes to NSPs is widely accepted. Xylanase in particular cleaves and hydrolyzes the xylose backbone of arabinoxylans, allowing endogenous enzymes access to entrapped nutrients, such as starch and protein, and increasing overall diet utilization by the bird (Choct and Annison, 1992; Bedford, 1996; Coweison et al., 2005; Meng et al., 2005). Because of this energy releasing effect, the use of xylanase in energy reduced corn-SBM rations has been extensively researched. Energy can be removed from diets through the reduction of dietary fat and increasing corn content of the diet (Masey O'Neill et al., 2012). Reducing the energy content of a diet is detrimental to broiler performance, with overall reductions in performance parameters (Cowieson et al., 2010; Masey O'Neill et al., 2012; Singh et al., 2012; Williams et al., 2014) and reductions in ileal digestibility (Cowieson et al., 2010; Yegani and Korver, 2013). The use of xylanse in energy reduced diets has resulted in improvements to FCR (Cowieson et al., 2010; Masey O'Neill et al., 2012; Williams et al., 2014) and increases ileal digestibility of energy, amino acids, and dry matter (Cowieson et al., 2010; Nian et al., 2011a; Yegani and Korver, 2013).

A substantial amount of research utilizing xylanase in wheat based diets has occurred over the past three decades. Wheat diets have been reported to contain more than twice the soluble NSP concentration than corn diets (Kiarie et al., 2014). The effectiveness of xylanase in these diets has been attributed to the higher substrate concentration in the diet, allowing for greater impact of xylanase supplementation in these diets as compared to corn based diets (Kiarie et al., 2014). Previous research by the authors has been conducted utilizing a novel xylanase in diets containing wheat and DDGS at 30 and 15%, respectively, and pelleted at increased temperatures (Pieniazek et al, 2013, 2014). When pelleted at 82°C, xylanase inclusion at 1000 and 2000 U/kg improved body weights during the grower phase. Xylanase inclusion at 250 U/kg and higher decreased cumulative FCR at the end of the 38 d experiment, while increasing d 38 ileal digestibility of energy (Pieniazek et al., 2013). A second experiment conducted by the authors utilized the same diet formulation pelleted at 92°C. Inclusion of xylanase at 250 U/kg and higher reduced cumulative FCR on d 21, and ileal digestible energy was increased with the supplementation of xylanase at 500 U/kg and higher (Pieniazek et al., 2014). Xylanase had no impact on BW at the conclusion of both experiments (Pieniazek et al., 2013 and 2014). Pelleting wheat diets at temperatures over 60°C has been reported to impact broiler performance through increases in diet and digesta viscosity (Abdollahi et al., 2010). Increases in previously encapsulated NSP has been observed when pelleting wheat based diets at elevated temperatures, resulting in overall decreases in performance parameters (Coweison et al., 2005; Abdollahi et al., 2010). Increasing conditioning temperature in corn based diets has also been shown to impact broiler performance by increasing FCR, although starch digestibility was unaffected (Abdollahi et al., 2010). The objective of this experiment was to determine the effects of increasing xylanase inclusion in reduced energy diets on observed growth performance, ileal digestibility of energy, relative organ weight, and processing parameters in diets manufactured at 85°C.

Materials and Methods

Experimental Design. A 43 d growout experiment was conducted to evaluate the effect of xylanase in an energy reduced corn-SBM diet. On d of hatch, 1,470 male broilers were weighed, wing banded, and allotted to floor pens and dietary treatments based on body weight. Thirty-five broilers were placed per replicate pen, with seven replicate pens per treatment, for a total of 42 replicate pens. Stocking density was set to 1.0 sq. ft./bird. The experiment was conducted under an approved Texas A&M Animal Use Protocol (IACUC). Broilers were provided age appropriate heat and ventilation and given access to feed and water *ad libitum*.

Broilers and feed were weighed on d 18, 35, and 42 (at the conclusion of each dietary phase) for the calculation of body weights (BW) and mortality corrected feed conversion ratio (FCR). On d 42, individual bird weights were recorded for each replicate pen. The coefficient of variance for each pen was calculated using individual bird weights. Liveability of broilers was calculated per replicate pen at after each period. A performance index (PI) was calculated utilizing these measurements after each phase and cumulatively using a modified equation by De Herdt et al. (1999):

$$\frac{(\text{Liveability (\%) x weight gain (kg)) x 10}}{\text{FCR}}$$

On d 18, one broiler per replicate pen was randomly selected and euthanized. Birds were necropsied and the proventriculus, gizzard, and pancreas were removed and weighed. Gizzard and ceca contents were collected and weighed for the determination of dry matter (dm) concentration. Samples were weighed and dried at 100°C for 24 hours. Dry matter was calculated by wet weight less dry weight divided by wet weight times 100. On d 39 litter samples were collected from each replicate pen for dm analysis. Samples were dried at 100°C for 24 hours, with dm calculated as stated above. On d 42, three birds per replicate pen were randomly selected and euthanized. Jejunum contents were collected and pooled per replicate pen for the determination of jejunum viscosity. Ileal samples were also collected and pooled for the determination of ileal dm and ileal digestibile energy (IDE).

Processing Parameters. On d 43, five broilers per replicate pen were randomly selected for processing. Feed was withdrawn from broilers 10 hours prior to processing. Live weights were recorded before transportation to the processing facility. Birds were killed via stun and serration of the jugular. Internal organs were removed and hot WOG and fat pad weights were recorded. Carcasses were immersion chilled to an internal temperature of 4°C. Following chilling, carcasses were weighed and deboned, with weights taken on the pectoralis major and minor for calculation of total breast meat yield.

Experimental Diets. Broilers were fed an industry type dietary program meeting the nutrient specifications outlined in Table 5-1. A standard corn-SBM diet was formulated and manufactured to be used as a positive control (PC). A negative control (NC) basal diet was formulated with a 100 kcal/kg AME reduction from the PC. One large NC basal diet was manufactured for each dietary phase to eliminate nutrient variability between treatments and divided into five equal parts. Titanium dioxide was included at 4% of the finisher diet, at the expense of corn, and utilized as an indigestible marker for the determination of IDE.

The xylanase enzyme evaluated was originally isolated from an environmental soil sample. The wild-type enzyme is a thermophilic glycoside hydrolase family (GH) 11 xylanase with a melting temperature (T_m) of 76°C (measured by differential scanning calorimetry). This wild-type xylanase was further optimized using directed evolution technology to create a hyper-thermostable variant with a T_m > 100°C. Premixes (400 g/MT) containing sand and enzyme were added to each dietary treatment prior to pelleting, with xylanase included at levels of 0, 250, 500, 1,000, and 2,000 U/kg. One unit (U) of activity is defined as 1 μmole of hydrolyzed xylan substrate per minute at 50°C and pH 5.3. Mash and pelleted samples of all diets and treatments were collected and analyzed for nutrient content (Table 5-1) and xylanase recovery (Table 5-2). Crude protein was determined by combustion using an AOAC (AOAC, 2000) method (AOAC

Table 5-1. Calculated and analyzed nutrient content and ingredient profiles of the two basal diets, positive control (PC) and negative control (NC) for the starter (d 1-18), grower (d 18-34), and finisher (d 34-42) dietary phases.

Ingredient Profile	Starter (%)		Grower (%)		Finisher (%)	
	PC	NC	PC	NC	PC	NC
Corn	56.75	59.26	62.35	64.89	67.41	69.94
Soybean Meal (48%)	35.16	34.72	30.25	29.78	25.34	24.88
A/V Fat	3.10	1.02	3.03	0.94	2.92	0.84
Limestone	1.73	1.73	1.53	1.54	1.41	1.41
Sodium chloride	0.46	0.46	0.39	0.39	0.28	0.28
Mono-calcium PO_4	1.80	1.79	1.40	1.39	1.14	1.14
Sodium bicarb	-	-	0.09	0.10	0.25	0.26
L-Lysine HCL	0.17	0.18	0.17	0.18	0.15	0.16
DL-Methionine (98%)	0.32	0.32	0.27	0.27	0.20	0.20
L-Threonine (98%)	0.07	0.07	0.07	0.07	0.05	0.05
Vitamins[1]	0.25	0.25	0.25	0.25	0.25	0.25
Trace Minerals[2]	0.05	0.05	0.05	0.05	0.05	0.05
Sand	0.10	0.10	0.10	0.10	0.10	0.10
Sacox 60[3]	0.05	0.05	0.05	0.05	0.05	0.05
Titanium dioxide	-	-	-	-	0.40	0.40
Calculated Nutrient Content						
Protein	22.28	22.28	20.34	20.34	18.35	18.35
Metabolizable Energy (kcal/kg)	3040	2940	3100	3000	3150	3050
Calcium	1.05	1.05	0.90	0.90	0.80	0.80
Phosphorus	0.75	0.76	0.66	0.66	0.59	0.59
Available Phosphorus	0.50	0.50	0.41	0.41	0.35	0.35
Methionine Total	0.65	0.65	0.58	0.58	0.48	0.48
Lysine Total	1.32	1.32	1.19	1.19	1.04	1.04
Threonine Total	0.90	0.90	0.82	0.82	0.73	0.73
Crude Fat	5.59	3.61	5.68	3.70	5.73	3.76
Sodium	0.20	0.20	0.20	0.20	0.20	0.20
Analyzed Nutrient Content						
Moisture	11.92	11.97	12.28	12.13	11.97	12.14
Crude Protein	21.3	21.5	19.3	18.2	17.8	18.4
Crude Fat	5.38	3.41	5.51	4.29	5.20	3.55
Acid Detergent Fiber	3.8	3.3	3.6	2.2	3.1	3.5
Ash	5.26	5.40	4.62	4.72	4.64	4.31

[1] Vitamin premix added at this rate yields 11,023 IU vitamin A, 3,858 IU vitamin D_3, 46 IU vitamin E, 0.0165 mg B_{12}, 5.845 mg riboflavin, 45.93 mg niacin, 20.21 mg d-pantothenic acid, 477.67 mg choline, 1.47 mg menadione, 1.75 mg folic acid, 7.17 mg pyroxidine, 2.94 mg thiamine, 0.55 mg biotin per kg diet. The carrier is ground rice hulls.

[2] Trace mineral premix added at this rate yields 149.6 mg manganese, 125.1 mg zinc, 16.5 mg iron, 1.7 mg copper, 1.05 mg iodine, 0.25 mg selenium, a minimum of 6.27 mg calcium, and a maximum of 8.69 mg calcium per kg of diet. The carrier is calcium carbonate and the premix contains less than 1% mineral oil.

[3] Active drug ingredient salinomycin, 60 g/lb (60 g/ton inclusion; Intervet Inc., Millsboro, DE). As an aid in the prevention of coccidiosis caused by *Eimeria tenella*, *Eimeria necarix*, *Eimeria acervulina*, *Eimeria maxima, Eimeria brunetti*, and *Eimeria mivati.*

method 990.03), total phosphorus was determined by wet ash inductively coupled plasma spectroscopy (AOAC method 985.01M), ADF was determined using an Ankom digestion unit (AOAC method 973.18) (Ankom Technology, Macedon, NY), and an ether extraction method was used to determine crude fat (AOAC method 920.39). The activity of the xylanase was determined by analyzing the amount of reducing sugars during enzymatic hydrolysis of xylan from beechwood. Activity was determined spectrophotometrically following color development with 3,5-Dinitrosalicylic Acid (DNS) buffer and compared to a xylose standard curve.

Broilers were fed a starter (d 1-18), grower (d 18-35), and finisher (d 35-43) diet. All diets were pelleted at 85°C with a 30 second conditioning time, with the starter feed crumbled post pelleting.

Viscosity. Viscosity of jejunum digesta was determined using the methods described by Lee et al. (2003). On d 42, three birds per replicate pen were randomly selected and necropsied. Jejunum contents were collected and pooled on a per pen basis. Digesta samples were centrifuged at 3,500 x g for 10 min. Supernatant (0.5 mL) was removed and placed in a Brookfield Cone and Plate Viscometer with a CPE-40 spindle at 40°C. Centipoise readings were taken after measuring for 30 seconds at 5 rpm.

Ileal Digestible Energy (IDE). IDE was determined at the conclusion of the experiment using titanium dioxide as an indigestible marker. Ileal contents were removed four centimeters posterior to Meckle's Divereticulum and four centimeters anterior to the ileal-cecum junction and pooled per replicate pen. Ileal and feed samples

Table 5-2. Xylanase recovery of corn-SBM diets manufactured at 85°C.

Treatment	Target Dosage (U/kg)	Feed Sample	Starter	Grower	Finisher
Positive Control (PC)	-	Mash	0	0	0
		Pellet	0	0	0
Negative Control (NC)	-	Mash	0	0	0
		Pellet	0	0	0
NC + 250 U/kg	250	Mash	451	362	370
		Pellet	121	171	115
NC + 500 U/kg	500	Mash	468	469	590
		Pellet	329	482	499
NC + 1,000 U/kg	1,000	Mash	825	1046	932
		Pellet	714	752	903
NC + 2,000 U/kg	2,000	Mash	1883	2155	1642
		Pellet	1748	1513	2053

were dried at 100°C for 24 hours. Samples were ground for gross energy and titanium concentration determination.

Titanium concentration was determined using a modified protocol outlined by Short et al. (1996). Half a gram of each dried sample was weighed and placed in an ashing oven at 450°C. Following ashing, each sample was titrated with 10 mL of 7.4 M sulfuric acid and boiled at 200°C for 3 hours until dissolved. Samples were then titrated with 10 mL of 30% hydrogen peroxide. Total sample volume of 100 mL was achieved using distilled water. Samples were analyzed for absorption using a Thermo Fisher Scientific Genesys 10S UV-Vis (Model 10S UV-Vis) Spectrophotometer at 410 nm. Gross energy of feed and ileal samples was determined using a Parr 6400 bomb calorimeter. IDE was calculated using the following equation (Scott et al., 1982):

$$\text{Gross } E_f - \text{Excreta } E_i \text{ where } E_i = GE \times (Ti_f/Ti_i)$$

Statistical Analysis

Body weights, liveability, mortality corrected FCR, PI, ileal dry matter IDE, IDEc, litter dry matter, relative organ weight, dry matter, and processing parameters were analyzed *via* Analysis of Variance (ANOVA) for completely randomized block design using the General Linear Model procedure using SPSS V 18.0. Data was subjected to one way ANOVA and means were deemed significantly different at $p<0.05$ and separated using the LS Means option. The experimental unit for each trait evaluated was pen.

Results

Performance. Overall, the reduction in energy between the PC and NC diets and the inclusion of xylanase in reduced energy corn-SBM diets had little impact on BW. On d 18, no impact was observed in average BW with the inclusion of xylanase or reduction in energy between the PC and NC diets (Table 5-3). The inclusion of xylanase in the energy reduced diet had no impact on BW on d 34 or 42 as compared to the energy reduced diet (NC), as all xylanase inclusion rates exhibited similar BW as the NC fed broilers. No differences in BW were observed between the PC and NC diets throughout the experiment indicating that the reduction in dietary energy was not sufficient to impact BW. Individual bird weighs were recorded on day 42 to allow for calculation of pen uniformity evaluated by coefficient of variation, but similar to the response in BW, no differences were observed between the PC and NC diets or with xylanase inclusion and the NC fed broilers.

Table 5-3. Body weights (BW), BW coefficient of variation (CV), dietary phase mortality corrected feed conversion ratio, and cumulative mortality corrected feed conversion ratio of broilers fed increasing levels of xylanase in corn-SBM diets.

Treatment	Body Weights			Day 42 CV	Dietary Phase FCR			Cumulative FCR	
	Day 18 (g)	Day 34 (kg)	Day 42 (kg)		Starter	Grower	Finisher	Day 1-34	Day 1-42
Positive Control (PC)	640.7	2.248[a]	3.113[a]	6.427[b]	1.408[ab]	1.643	1.831	1.577	1.655[b]
NC (PC - 100 kcal/kg)	632.6	2.187[ab]	3.067[ab]	7.597[ab]	1.437[a]	1.719	1.820	1.637	1.697[a]
NC + 250 U/kg	652.8	2.201[ab]	3.090[ab]	7.479[ab]	1.404[b]	1.700	1.783	1.613	1.670[b]
NC + 500 U/kg	640.6	2.166[ab]	3.009[b]	7.583[ab]	1.396[b]	1.706	1.843	1.614	1.687[a]
NC + 1000 U/kg	644.7	2.150[b]	3.019[ab]	8.147[a]	1.407[ab]	1.727	1.856	1.630	1.696[a]
NC + 2000 U/kg	628.5	2.163[ab]	3.037[ab]	7.819[ab]	1.402[b]	1.693	1.810	1.609	1.676[ab]
SEM	4.4	0.014	0.014	0.206	0.005	0.013	0.018	0.009	0.005

[a-c] Means in columns with different superscripts differ at $p \leq 0.05$.

With regards FCR, inclusion of xylanase at 250, 500, and 2,000 U/kg in the energy reduced diet significantly reduced ($p<0.05$) starter phase FCR as compared to the NC diet (Table 5-3). The reduction in dietary AME did not significantly impact grower and finisher diet FCR. Additionally, inclusion of xylanase in the energy reduced diet had no impact on grower and finisher phase FCR as compared to the NC diet. At the conclusion of the experiment, a significant reduction ($p<0.05$) in d 1-42 cumulative FCR was observed between the PC and NC diets as an energy reduction increased FCR. The inclusion of xylanase at 250 U/kg in the energy reduced diet reduced ($p<0.05$) cumulative FCR as compared to the NC to levels similar to the PC while inclusion at 2,000 U/kg was intermediate.

No differences in liveability were observed between the PC and NC diets throughout the experiment (Table 5-4) and all xylanase inclusion treatments were similar to the NC fed broilers.

The impact of dietary energy on FCR impacted calculated flock European Performance Index (PI). Although no differences in PI were observed in the starter, grower, and finisher phases between the PC and NC diets (Table 5-5), inclusion of xylanase at 250 U/kg in the energy reduced diet significantly increased ($p<0.05$) starter PI as compared to the NC diet. With regards to cumulative PI, decreasing dietary energy level reduced ($p<0.05$) d 1-34 PI with the NC diet having a lower PI than the PC diet. Inclusion of 250 U/kg increased d 1-34 PI to a level similar to the PC diet. Day 42 PI was decreased ($p<0.05$) with the reduction in the NC diet as compared to the PC fed broilers. Inclusion of xylanase at 250 U/kg increased d 42 PI to a level similar to the PC diet.

No differences in litter dm were observed between the PC and NC diets; litter dm was not impacted with the inclusion of xylanase (Table 5-6).

Digestive Organ Weights, Dry Matter Content, and Viscosity. Following the starter phase of production (d 18), no differences were observed in gizzard weight, relative gizzard weight, and gizzard content dm associated with energy reduction or xylanase inclusion (Table 5-7). No differences were observed in ceca dm between the PC and NC diets, however, the inclusion of xylanase at 1,000 U/kg in the energy reduced diet significantly reduced ($p<0.05$) ceca DM as compared to the PC and NC diets. No differences in proventriculus weight were associated with dietary energy level

Table 5-4. Dietary Phase percent liveability (PL; %) and cumulative PL for broilers fed increasing levels of xylanase in corn-SBM diets.

Treatment	Dietary Phase PL			Cumulative PL	
	Starter	Grower	Finisher	Day 1-34	Day 1-41
Positive Control (PC)	97.55[a]	99.15	100.00	96.73	96.73[a]
NC (PC - 100 kcal/kg)	95.92[ab]	100.00	99.51	95.92	95.51[ab]
NC + 250 U/kg	96.73[ab]	99.58	99.12	96.33	95.51[ab]
NC + 500 U/kg	94.29[b]	99.57	99.06	93.88	93.06[b]
NC + 1000 U/kg	97.55[a]	99.18	100.00	96.73	96.73[a]
NC + 2000 U/kg	94.29[b]	99.51	100.00	93.88	93.88[ab]
SEM	0.52	0.17	0.17	0.54	0.56

[a,b] Means in columns with different superscripts differ at $p \leq 0.05$.

Table 5-5. Period performance index (PI) and cumulative performance index for broilers fed increasing levels of xylanase in corn-SBM diets.

Treatment	Period PI			Cumulative PI	
	Starter	Grower	Finisher	Day 1-34	Day 1-42
Positive Control (PC)	417[ab]	971[a]	474	1356[a]	1796[a]
NC (PC - 100 kcal/kg)	395[b]	906[ab]	486	1258[b]	1703[bc]
NC + 250 U/kg	423[a]	911[ab]	496	1294[ab]	1745[ab]
NC + 500 U/kg	406[ab]	894[ab]	455	1239[b]	1637[c]
NC + 1000 U/kg	419[ab]	870[b]	483	1256[b]	1700[bc]
NC + 2000 U/kg	397[b]	904[ab]	484	1239[b]	1680[bc]
SEM	4	13	11	15	14

[a-c] Means in columns with different superscripts differ at $p \leq 0.05$.

Table 5-6. Litter dry matter for broilers fed increasing levels of xylanase in corn-SBM diets.

Treatment	Litter Dry Matter %
Positive Control (PC)	63.55
NC (PC - 100 kcal/kg)	65.91
NC + 250 U/kg	64.26
NC + 500 U/kg	65.69
NC + 1000 U/kg	65.44
NC + 2000 U/kg	69.04
SEM	0.86

Table 5-7. Relative organ weights, digesta dry matter (DM) percentages, and jejunum viscosity (cP) for broiles fed increasing levels of xylanase in corn-SBM diets.

Treatment	Gizzard (g)	Gizzard % BW	Gizzard DM (%)	Ceca DM (%)	Provent. (g)	Pancreas (g)	Pancreas % BW	Jejunum Viscosity (cP)
Positive Control (PC)	15.80	2.55	30.06	21.16[a]	0.684	2.09[ab]	0.34	1.407[ab]
NC (PC - 100 kcal/kg)	14.79	2.38	31.72	21.42[a]	0.622	2.09[ab]	0.33	1.766[a]
NC + 250 U/kg	16.69	2.46	33.20	19.26[ab]	0.629	2.19[a]	0.32	1.558[ab]
NC + 500 U/kg	15.60	2.48	33.16	20.70[a]	0.624	2.16[a]	0.34	1.646[ab]
NC + 1000 U/kg	16.26	2.61	30.06	16.76[b]	0.638	1.99[ab]	0.33	1.402[b]
NC + 2000 U/kg	14.79	2.44	31.67	20.68[a]	0.580	1.67[b]	0.28	1.528[ab]
SEM	0.29	0.04	0.56	0.50	0.020	0.07	0.01	0.053

[a,b] Means in columns with different superscripts differ at $p \leq 0.05$.

or xylanase inclusion. No difference in pancreas weight and relative pancreas weight was observed between the PC and NC diets. Although inclusion of xylanase in NC diet had no impact on pancreas weight and relative pancreas weight as compared to the control diets, inclusion of xylanase at 2,000 U/kg significantly reduced ($p<0.05$) pancreas weight as compared to supplementation of xylanase at 250 and 500 U/kg.

With regards to d 42 jejunum viscosity, no difference in jejunum viscosity was observed between the PC and NC diets (Table 5-7). Inclusion of xylanase at 1,000 U/kg significantly reduced jejunum viscosity as compared to the NC diet although viscosity measurements were low which was expected with a corn-SBM diet.

Ileal Digestible Energy. No differences in ileal dm were associated with energy reduction or xylanase inclusion (Table 5-8). IDE was reduced ($p<0.05$) in the NC fed broilers as compared to PC fed broilers. Inclusion of xylanase at 250, 500, and 2,000 U/kg in the reduced energy NC diet increased IDE to a level comparable to the PC fed broilers.

Table 5-8. Ileal DM (%),IDE for broilers fed increasing levels of xylanase in corn-SBM diets.

Treatment	Ileal DM (%)	IDE
Positive Control (PC)	21.67	3205^{a}
NC (PC - 100 kcal/kg)	21.51	3047^{b}
NC + 250 U/kg	21.88	3112^{ab}
NC + 500 U/kg	21.26	3082^{ab}
NC + 1000 U/kg	21.27	3008^{b}
NC + 2000 U/kg	21.69	3113^{ab}

^{a,b} Means in columns with different superscripts differ at $p \leq 0.05$.

Processing Parameters. On d 43, no differences in live, hot carcass without giblet (WOG), abdominal fat pad, chilled WOG, and breast weight were observed between the PC and NC diets as well xylanase inclusion did not result in differences between the NC and NC with xylanase inclusion at any level (Table 5-9). The reduction in energy resulted in no differences in carcass, fat pad, and breast yield between the PC and NC diets. The inclusion of xylanase in the reduced energy diet had no impact on carcass and fat pad yield compared to the NC diet. However, broilers fed the diet with xylanase inclusion at 250 U/kg in the NC diet exhibited increased ($p<0.05$) breast yield as compared to the NC diet.

Table 5-9. Processing data for broilers fed increasing levels of xylanase in corn-SBM diets.

Treatment	Live Wt (g)	Hot Wog Wt (g)	Fat Pad Wt (g)	Wet WOG Wt (g)	Breast Wt (g)	Wog Yield (%)	Fat Pad %	Breast %
Positive Control (PC)	3076	2317	38.9	2336	668.5	75.31^{ab}	1.68	28.60^{ab}
NC (PC - 100 kcal/kg)	3124	2350	43.3	2378	675.4	75.24^{ab}	1.84	28.40^{b}
NC + 250 U/kg	3106	2347	40.4	2370	700.0	75.57^{ab}	1.72	29.53^{a}
NC + 500 U/kg	3062	2291	39.6	2317	662.5	74.81^{b}	1.73	28.61^{ab}
NC + 1000 U/kg	3046	2304	39.4	2318	670.5	75.66^{a}	1.70	28.92^{ab}
NC + 2000 U/kg	3083	2319	39.2	2325	675.3	75.24^{ab}	1.68	29.05^{ab}
SEM	19	15	0.8	15	5.6	0.12	0.03	0.14

^{a,b} Means in columns with different superscripts differ at $p \leq 0.05$.

Discussion

With a large portion of diet cost incurred due to dietary energy levels, nutritionists have been charged with the task of lowering dietary energy without impacting broiler performance. Diets can be formulated for reduced energy content through reductions in dietary fat inclusion and increasing corn content (Masey O'Neill et al., 2012). However, reductions in AME results in decreased BW (Singh et al., 2012), carcass yields (Singh et al., 2012), and increased FCR (Cowieson et al., 2010; Masey O'Neill et al., 2012; Williams et al., 2014). These observations support the findings of the current experiment, where a 100 kcal/kg decrease in AME resulted in a 4.2 point increase in cumulative FCR from d 1-42. The reduction in AME during the current experiment had no impact on BW throughout the experiment. Observed increases in feed consumption, and thus FCR, has been explained by Cowieson et al. (2010) as to how BW gains of broilers fed energy reduced diets can match those of standard AME diets. IDE was reduced by nearly 5% on d 42 of the current experiment with the reduction in dietary energy. This observation was previously reported by Cowieson et al. (2010) when AME was reduced by 110 kcal/kg.

Though low in NSP concentration, corn-SBM diets have the potential to be maximized through the supplementation of exogenous enzymes. Corn starch content is relatively high in comparison to most cereal grains (NCGA, 2013). However, Noy and Sklan (1994) report 15% of corn starch goes undigested by broilers, limiting the energy value of corn in broiler diets. Because arabinoxylans entrap corn starch, rendering it unavailable for use to the bird (Gracia et al., 2003; Cowieson, 2005; Choct, 2006; Slominksi, 2011), inclusion of exogenous xylanase could provide for a release of entrapped starch, and thus available energy, once unavailable to the bird (Cowieson, 2010). This would ideally allow for AME reductions in diet formulations with no impact on broiler performance.

In the current study, the inclusion of xylanase at the lowest dosage of 250 U/kg in the energy reduced diet had no impact on BW as compared to the control diets. However, increasing xylanase dosage to 500 and 1000 U/kg was unable to overcome the reduction in dietary ME, resulting in reduced BW of 4.4% in the grower and 3.3% in the finisher, respectively, compared to the PC. This occurrence was unexpected, as in the previous studies conducted by Pieniazek et al. (2013, 2014) the novel xylanase had no impact on BW when included in a wheat-DDGS diet, suggesting variations in xylanase dose response in corn-SBM diets as opposed to viscous grain diets. Inconsistencies in BW response with this product reinforce the issue of utilizing exogenous enzymes in that they may not always benefit or enhance growth performance (Cowieson et al., 2006), in this case unable to overcome a reduction in A ME. Although no impact in BW at a high dose was observed, no other dosage level of xylanase had an impact on broiler BW as compared to the standard and energy reduced diet, in line with observations by Masey O'Neill et al. (2012), Singh et al. (2012), and Williams et al. (2014).

Although no improvements in BW were observed with xylanase, supplementation of the energy reduced diet decreased starter phase FCR by a range of 3.3-4.1 points to levels similar to, and numerically lower, than the PC, suggesting xylanase supplementation influenced performance through a reduction in feed intake. No differences in FCR were observed in later phases of production. Olukosi et al. (2007) suggested that a greater response to enzymes should be expected in younger birds versus older due to the undeveloped and limited endogenous enzyme activities in the digestive tract. Kiarie et al. (2014) also state that young birds are sensitive to both soluble and insoluble NSP, allowing for benefits of xylanase in all diets regardless of grain type. The significant impact made by xylanase inclusion during the starter phase were significant enough to impact cumulative FCR at d 42 when xylanase was included in the diet at 250 U/kg, although no significant differences were observed during other dietary phases. These findings are supported by Williams et al. (2014), who observed reductions in FCR by xylanase inclusion at d 15 and then cumulatively at d 42. The early impact of FCR and its influence on final cumulative FCR in the current study is opposite of the overall impact of xylanase observed by Masey O'Neill et al. (2012), who reported an improvement of xylanase over the lifetime of the bird, suggesting the impact of xylanase develops with age due to the overall impact on digestive microorganisms. The key finding of this experiment though was the reduction in FCR only by the lowest dosage of xylanase in the energy reduced diet, suggesting only a small amount of xylanase activity may be needed to improve FCR when broilers are fed corn-SBM diets.

The European Performance Index (PI) was calculated to further evaluate the differences in impact of xylanase supplementation in the energy reduced diets. This calculation brings together all performance variables typically reported in literature, including BW, FCR, and percent livability. Xylanase inclusion from 250 to 1000 U/kg in the starter phase improved PI to levels similar to the PC, however, only the lowest inclusion rate of xylanase was able to significantly raise PI above the NC. This trend appeared once again in the final d 42 cumulative PI, with xylanase inclusion at 250 U/kg increasing PI 2.5% as compared to the NC while no other inclusion rate was able to overcome the reduction in energy. Because the calculation of PI is dependent on FCR, without differences in liveability and body weight gain, it is possible to presume FCR had the greatest impact on the calculation of PI in the current study. It could also be presumed that PI could be a useful tool in defining and separating observed differences when evaluating exogenous enzyme supplementation, as many times improvements in BW and FCR are observed but, individually, cannot statistically separate from the NC.

Reductions in digesta viscosity have been strongly attributed to the benefits of xylanase in viscous grain diets, such as wheat. Nian et al. (2011b) attributes the greater benefit of xylanase in wheat due to the increased amounts of soluble NSP in wheat (24 g/kg) as compared to corn (1g/kg) (Choct, 1997). Although a difference in jejunal viscosity of wheat and corn based diets was reported by Kiarie et al. (2014), the group reported similar viscosity levels of with the supplementation of xylanase, regardless of diet type. In the current experiment, supplementation of xylanase at 1000 U/kg significantly reduced jejunum viscosity (20.6%) as compared to the reduced energy diet, with numerical improvements at all other doses. The use of supplemental xylanase to free bound starch from arabnioxylans is typically thought to be a mechanism for improvements in digestibility and overall performance in corn-SBM diets (Choct and Annison, 1992; Bedford, 1996; Coweison et al., 2005; Meng et al., 2005). In the present study, the reduction of dietary AME reduced IDE in the NC as compared to the PC diet. Inclusion of xylanase in the NC improved IDE at d 42 to comparable levels of the PC fed broilers. Yegani and Korver (2013) reported no change in finisher IDE in xylanase supplemented diets containing various corn sources, although an increase in IDE was reported by the group during the grower phase. While the findings of the current study do indicate IDE improvements to levels of the PC, the significant improvements in IDE as compared to the NC were not observed with xylanase inclusion. These results differ from previous experiments by the authors utilizing the novel xylanase, although the diet included wheat and DDGS (Pieniazek et al., 2013, 2014). The different results between these experiments is due to the presence or absence of alternative ingredients such as wheat and DDGS and confirms that that energy release associated with xylanase inclusion is substrate dependent and more pronounced as NSP content of the diet increases.

Other benefits to digestive tract parameters due to xylanase inclusion in diets have been observed. The current study investigated dm content of specific digestive organ digesta in the starter and finisher phases. There was no influence of energy content or xylanase supplementation on gizzard dm content on d 18. However, dm content was reduced as much as 4.66 percentage points in the ceca with the addition of xylanase at 1000 U/kg in the energy reduced diet as compared to the PC and NC. Numerical reductions in ceca dm content ($p = 0.088$) were also observed with all other inclusion levels of xylanase. Although ceca dm content was reduced on d 18, ileal dm content was unaffected by dietary energy content or supplementation of xylanase on d 42. Litter dm content was also unaffected on d 39. Feed and digesta viscosity have been linked to increases in water consumption in broilers fed viscous grains, lowering water absorption and increasing water loss in the excreta (Francesch and Brufau, 2004). Without a significant biological effect on digestive viscosity observed, it is reasonable to assume that dm content of the litter would not have been affected.

The reduction in dietary energy and supplementation of xylanase had no effect on d 18 gizzard and proventriculus weights. These findings contradict those of Sheng et al. (2013) in which xylanase supplementation increased gizzard growth in a corn-SBM diet, although not in an energy reduced diet. Interestingly the addition of xylanase at reduced pancreas weight in a linear trend, from 2.19g in the 250 U/kg xylanase supplemented diet to 1.67g in the 2000 U/kg supplemented diet and becoming significant, indicating a dosage effect of xylanase. Wang et al. (2005) also reported a linear relationship between increasing xylanase dosage and the relative weight of the pancreas in a wheat based diet. This effect has been suggested to also occur in corn-SBM diets (Nian et al., 2011b). Brenes et al. (1993) indicated that increases in digestive weights of diets not supplemented with enzymes suggested an adaptive response for the need of enzymes in a barley based diet. It could then be suggested that the reduction in pancreas weight could be an adaptive response to the reduced need of endogenous enzymes, indicating further action of xylanase beyond the targeted substrates in the gut.

Because carbohydrases have been shown to benefit performance, it is important to observe any final improvements xylanase may play in processing parameters. Coppedge et al. (2012) reported an increase in breast meat weight with the use of an NSPase complex containing 1500 U/kg xylanase along with β-glucanase, α-galactosidase, and β-mannanase. Although energy reduction had no impact on breast meat yield between the control diets, supplementation of xylanase at 250 U/kg to the energy reduced diet increased breast meat yield as compared to the NC. The current experiment observed no impact of energy reduction or xylanase inclusion on WOG carcass, fat pad, and breast meat weights. However, a dose response was observed in carcass yield, with xylanase inclusion of 1000 U/kg having a greater WOG yield than that of xylanase inclusion at 500 U/kg. A greater carcass yield in xylanase supplemented diets was also observed by Williams et al. (2014). Brenes et al. (1993) suggested that an increase in WOG yield should be expected with xylanase inclusion, as dressing yield is connected to the reduction in organ weights caused by xylanase inclusion in diets.

In conclusion, the use of supplemental xylanase at 250 U/kg in the reduced energy diet corrected for losses in starter and cumulative FCR and the performance index to levels similar to the PC. Reductions in ceca DM content, pancreas weight, and jejunal viscosity were also with varying inclusion rates of xylanase. Final breast meat yields were also improved with xylanase inclusion at 250 U/kg to the energy reduced diet. These data confirm the ability of xylanase to improve growth performance in a low energy corn-SBM diets through increases in IDE and indicate potential yield benefits associated with changes in organ weight although further understanding of dosage, dependent on ingredient profile, is warranted.

CHAPTER VI

CONCLUSION

The bulk of the cost associated with intensively reared broiler operations is feed cost and the prevention and mitigation of diseases. Feed cost accounts for the largest cost of production to broiler integrators. Disease outbreaks caused by *Eimeria spp.* and Newcastle Disease Virus can also account for extreme costs on producers (CIDRAP, 2003; Dalloul and Lillehoj, 2006; Shirley et al., 2006; McDonald and Shirley, 2009; Cox et al., 2010b) if not handled properly through proper prevention and management. Morbid broilers do not utilize feed efficiently, thus causing losses through poor performance and thus losses through low carcass weights and mortality. Chemotheraputic methods of managing disease outbreaks have been the method of choice for treating infected broilers in the past, with antibiotic growth promoters being the feed additive of choice by broiler producers to imrove growth performance and overall health of birds (Cox and Dalloul, 2010; Huyghebaert et al., 2011). These products allowed for immune mitigation, but were primarily used to benefit performance through reductions in FCR and improvements to BW. This practice was forced to discontinue with the ban of antibiotics in livestock and poultry by the EU in 2006 (Castanon, 2007). Increasing consumer pressure has also led to reductions and limitations on the use of antibiotics in the U.S. This research program focused upon new products as alternatives to antibiotics to benefit broiler growth performance through increased utilization of dietary ingredients and improvements to broiler immune status.

Concerns regarding resistance to antibiotics by pathogens, the incidence of antibiotic residues in meat, and the resulting ban on the use of these products, have emphasized the need for research to identify alternative feed additives to provide both benefits to immune function and to maintain and performance benefits associated with antibiotic growth promoters. Products composed of plant cell wall derived β-(1,3)-D-glucans have been researched as alternatives to antibiotics due to their immune stimulating effects. Supplementation of ABG in a non-challenge setting at 750 g/MT increased ($p<0.05$) d 14 BW 3.1% compared to the unsupplemented birds; supplementation of ABG at 250 g/MT decreased ($p<0.05$) mortality corrected FCR by 4.6 points during the starter phase. No impact by ABG was observed on relative organ weights in a non-challenge setting. The benefits to performance were replicated pre-*Eimeria* challenge in the second experiment through supplementation of ABG. Addition of ABG at 250 g/MT increased ($p<0.05$) d 10 BW 7.2% as compared to the control diets; supplementation of ABG at 250 g/MT and greater reduced ($p<0.05$) mortality corrected FCR over 7.3 points as compared to the control. These benefits continued cumulatively through the end of the experiment. No impact on intestinal lesion scores and oocyst output was detected with the supplementation of ABG, although a dose difference was observed between supplementation of ABG at 250 and 750 g/MT in both parameters. No impact by ABG was observed on performance during a Newcastle Disease Virus vaccine program. However, supplementation of ABG at 250 g/MT increased ($p<0.05$) Newcastle Disease specific antibody titers. The results of these experiments indicate that an algal derived β-(1,3)-glucan can benefit early growth performance in a non-challenge setting and stimulate the immune system during an *Eimeria* challenge and Newcastle Disease vaccine program.

Choice of ingredients used in broiler rations is dependent on the grains readily available in a region and at a low cost (Bedford, 1995). Wheat-based diets are predominantly used in areas where corn is low in availability and high in price. DDGS, a by-product of ethanol production, is a nutritionally available grain alternative with increasing availability correlated with the rise in corn usage for ethanol production (Swiatkiewicz and Koreleski, 2008). However, viscous grains such as wheat and DDGS contain high concentrations of non-starch polysaccharides, whose antinutritive effects negatively impact broiler performance (Henry, 1984; Choct and Annison, 1990; Annison, 1993; Cromewell et al., 1993; Belyea et al., 2004; Swiatkiewicz and Koreleski, 2008; Caprita et al., 2010; Kiarie et al., 2014). The use of carbohydrases to degrade NSPs has been suggested to offset the antinutritive effects of NSP. In the current experiment, supplementation of xylanase in diets containing wheat and DDGS pelleted at 82 and 92°C had no impact on final BW. However, the addition of xylanase at 250 U/kg and higher decreased ($p<0.05$) cumulative mortality FCR by a range of 6.1 to 8.0 and 5.4 to 15.2 points in diets pelleted at 82 and 92°C, respectively. The reductions in FCR were attributed to the reductions ($p<0.05$) in feed consumption observed in broiler diets supplemented with xylanase. An increase ($p<0.05$) in ileal digestibility of energy was also observed in diets supplemented with xylanase at 500 and greater at both pelleting temperatures. The results of these experiments indicate that this particular experimental xylanase can provide benefits to performance through the release in digestible energy in diets containing wheat and DDGS.

Corn is the most abundant cereal grain on a global scale, and is used as the bulk source of energy for poultry diets (Cowieson, 2005). However, 400-450 kcal/kg of ME goes undigested and thus unutilized by broilers (Cowieson, 2010). This phenomenon has been attributed to the relatively low, but still present, levels of NSP that entrap corn starch and protein. The use of supplemental xylanase may allow for even greater utilization of corn starch by broilers, although 100% digestibility is unlikely (Cowieson, 2010). Xylanase may prove beneficial to corn-based diets, allowing for reductions in dietary energy through the replacement of fat with corn, lowering diet cost via a lower cost for energy, while still maintaining growth parameters. The use of xylanase in the current research at 250, 1000, and 2000 U/kg in the energy reduced diet was able to maintain similar final BW to the standard energy diet. Early performance benefits were also observed with mortality corrected FCR in the starter phase, with xylanase inclusion at 250, 500, and 2000 U/kg in the energy reduced diet reducing ($p<0.05$) FCR as compared to the unsupplemented control diet at a range of 3.3 to 4.1 points. No impact in xylanase or dietary energy was observed in the grower or finisher stages. However, supplementation of xylanase at 250 U/kg in the energy reduced diet reduced ($p<0.05$) d 1-42 cumulative FCR 2.7 points, to a level not different from the standard energy diet. The calculation of the European Performance Index amplified these results, showing the overall impact of xylanase supplementation at 250 U/kg, with the lowest dosage being the only level of supplementation to have a similar performance index to the standard energy diet. No impact on ileal digestibility of energy was observed between the control diets and diets supplemented with xylanase, although a reduction in jejunal viscosity was observed with the supplementation of xylanase at 1000 U/kg as compared to the unsupplemented energy reduced diet. A reduction in ceca dry matter percentage was also observed with xylanase supplementation at 1000 U/kg as compared to the control diets. Xylanase supplementation had no impact on litter dry matter, relative gizzard weight, relative pancreas weight, and gizzard dry matter percentage. With regards to processing parameters, the addition of xylanase to the energy reduced diet had no impact on WOG weight, fat pad weight and yield, and breast meat weight. However, inclusion of xylanase at 250 U/kg in the energy reduced diet increased ($p<0.05$) breast meat yield 1.13 percentage points as compared to the unsupplemented control diet. A dosage effect in xylanase supplementation at 500 and 1000 U/kg was also observed with regards to carcass yield. These data suggest that xylanase supplementation at a low dosage level can benefit performance and processing parameters in energy reduced diets.

Combined, these data indicate the ability of a novel algal derived β-(1,3)-D-glucan on early performance of non-challenged broilers and stimulate the immune system during an *Eimeria* challenge and a Newcastle Disease vaccine program. Subsequently, these data also indicate the benefit of an experimental xylanase on broiler performance in diets containing wheat and DDGS at 30 and 15%, respectively, when pelleted at 82 and 92°C; the data also indicate the benefit of low xylanase dosage on performance in a standard corn-SBM diet when pelleted at 85°C fed to broilers for 42 d.

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